# Ciprofloxacin Therapy for Neonates and Young Infants:

# A Clinical Pharmacological Perspective including Pharmacokinetics (PK) and Pharmacodynamics (PD)

Towards licensing this antimicrobial for neonates and young infants within the European Regulatory Framework

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by:


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# Abstract

**Ciprofloxacin Therapy for Neonates and Young Infants:**

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Severe infection due to Gram-negative bacteraemia increases mortality and long term consequences of morbidity. In Liverpool Women's NHS FT neonatal unit mortality increased from 20% for the more premature babies to 44%. Ciprofloxacin is a synthetic fluoroquinolone prescribed for suspected or proven Gram-negative infection in many neonatal units throughout Europe (25%). At present this drug is not authorised for this population and is prescribed off label. The optimal drug regimen is unknown resulting in a wide range of regimens (5 to 60 mg/kg/day) prescribed internationally. The TINN Consortium 'Treat Infection in Neonates' were funded by a European Union FP7 grant to develop a Paediatric Investigation Plan in line with the EU Paediatric Regulations and European Medicines Agency requirements. This thesis describes a prospective population PK clinical trial as part of the work of TINN, a retrospective clinical outcome study in neonates and infants with late onset Gram-negative sepsis and a framework to facilitate PK-PD research.

The pharmacokinetic (PK) clinical trial of ciprofloxacin recruited 64 babies including a minimum of seven recruits representing every four weeks of development. The median post menstrual age in weeks was 35.7 (6.5) (range 24.9 - 47.9). Blood samples (n = 265) were collected at pre-defined informative time points in addition to 165 samples scavenged from clinically indicated blood tests, in total 430. A two compartment model with first-order elimination fitted the data. PK parameters included maximum concentration, clearance, area under the curve (0-tau), apparent volume of distribution and half-life and selected covariates with their inter-individual variability (CV%). Based on adult PD targets the minimum area under the curve /minimum inhibitory concentration (AUC/MIC) ratio 125 was achieved by 85% of this

population. Higher $AUC_{24\ hour}$/MIC targets >250 may be required for serious infections yet few (42%) of this population achieved this. The optimal AUC/MIC ratio for neonates is unknown. Clearance increased with post-natal age yet the combined effect of PMA at birth and corrected gestational age had a greater effect on clearance. Clearance reduced by 29% with co-administration of inotropes associated with underlying renal compromise. Monte Carlo simulation demonstrated that the $AUC_{24}$/MIC 125 was achieved by 90% of neonates <34 weeks PMA administered 7.5 mg/kg/day and 84% of those ≥34 PMA administered 12.5 mg/kg/day. Ciprofloxacin is lipophilic and has good tissue penetration. CSF concentrations achieved a ratio of 0.33 of the serum plasma concentrations.

Pharmacodynamic data included a retrospective cohort study over a six year period of neonates with confirmed Gram-negative organisms in blood cultures. Organisms retrieved from the clinical laboratory (n=88) were re-cultured and the MIC ciprofloxacin determined using an E Test. A sub-group were administered ciprofloxacin (n=33); each organism's MIC was compared with the same neonate's clinical outcome. Relatively higher MICs within the susceptible range were associated with a greater risk of treatment failure. These data were not statistically significant due to the low incidence of confirmed Gram-negative bacteraemia (1.3%). The clinical implications are that more intensive dosage regimens may be required or a lower clinical breakpoint subject to further PD and safety data. Too few organisms were available to determine if the MIC increased annually but the incidence of ciprofloxacin resistance in surveillance surface swabs was no higher than for gentamicin.

To prepare for a future pharmacogenomic study, DNA scavenged from clinical blood samples were compared to a buccal scrape to evaluate whether this less invasive method is reliable. A substantially larger quantity and higher quality of DNA was obtained from scavenged blood. A sub-group were transfused with leukocyte depleted products for their clinical care prior to DNA sampling. The genotyping of allelic

polymorphisms were not affected by the donor's blood sufficiently to affect the genetic fingerprint. This indicates that prior blood transfusions should not be a contra-indication to scavenging blood for pharmacogenetic purposes.

The challenges of PK/PD trials in this vulnerable population were explored. The majority were recruited in intensive care and had comorbidity and extreme prematurity. Due to the complexity of critical illness there are limitations to interpreting clinical outcome or safety data from a PK clinical trial design. There were no suspected unexpected serious adverse reactions. Arthralgia was not reported but is difficult to assess in non-weight bearing non-vocal neonates and young infants. A proportionate regulatory model of neonatal PK clinical trials was developed with the Medicines and Health Care Products Regulatory Agency. A framework for pharmacovigilance reporting during critical illness which avoided nuisance reporting was developed. The impact was assessed by an observational case study and review of discharge letters to illustrate the challenge of attributing causality.

Individualised therapy may be required in critical illness. The correct dose of an antimicrobial may change daily during the sepsis episode. The inter- and intra-individual variability in parameters is associated with dynamic changes during neonatal development and the effects of critical illness. Further outcome and safety data are required to determine the optimal $AUC_{24}$/MIC ratio. Paediatric clinical breakpoints specific to each sub-age group of children are required. Individualised therapy may be required to optimise clinical outcome and minimise resistance.

# Declaration

No portion of this work has been submitted in support of an application for degree or qualification of this or any other University or institute of learning.

The clinical trial performed as part of this thesis was sponsored by Liverpool Women's NHS FT LW0852 and approved by both the Medicines and Health Care Products Agency (MHRA) EudraCT number 2010-01995523, the National Research Ethics Service reference number 10/H1002/79 and registered with the NIHR UKCRC 9508. Informed consent was given by parents for the Pharmacokinetic Clinical Trial.

The retrospective microbiology and clinical outcome study were approved by the National Research Ethics Service reference 10/H101/48.

# Dedication

The thesis is dedicated to the neonates or young infants and their families who took part and made the clinical trial possible. At a particularly stressful and emotional time of their own lives they kindly supported this research to benefit other families and children.

This thesis aims to find clinical answers to improve the care of neonates and young infants. Over several years of caring for children in diverse settings including paediatric intensive care units and developing countries the burden of infection is evident. Many of these children suffered severe infection as a consequence of disease, trauma including major burn injury, prematurity and congenital abnormalities. Infection added to what they had to endure due to their underlying health condition often with fatal consequence. Preventing and optimising the treatment of infection is of great benefit. Some of these children were admitted to intensive care for over a year exposed to repeated episodes of infection. The thesis is dedicated to these children and others who inspired the research:

*Angela*

*Aaron*

*Elize*

*James*

*R'Sharne*

*…and many others*

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- Dr Timothy Neal - Consultant Microbiologist
- Dr Jamie Kirkham - Statistician
- Professor Anthony Nunn
- Dr Mark Turner – Neonatal Senior Lecturer

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I appreciate the opportunity to undertake this PhD offered by Dr Mark Turner and Professor Tony Nunn. This was to support of my ambition to develop a new formulation of antimicrobial suppositories for children by developing my academic and clinical knowledge of neonatal and infant infection and pharmacology.

I am privileged that Professor Jason Roberts, University of Queensland and Royal Brisbane Hospital kindly contributed to the thesis and shared his expertise on the concepts of PKPD and optimisation of antibiotic dosing in critical illness.

Jo Drury has been exceptionally supportive in all aspects by reviewing each chapter and collaborating in the design and delivery of the DNA sample study. I would like to thank Mandy Hall, Christine Chesters and Kevin Padmore for their laboratory support and Dr Stephane Paulus, for guidance in paediatric infection. Kim Hall administrator who has been exceptionally supportive at all times. Finally, thanks to the, phlebotomists, nurses, pharmacists and medical staff at Liverpool Women's and Alder Hey Children's NHS FT who contributed to achieving the precision required and for supporting the families.

# Abbreviations

| | |
|---|---|
| ADC | Apparent Diffusion Coefficient |
| ADME | Absorption Distribution Metabolism Excretion |
| ALT | Alanine Transaminase |
| AMR | Antimicrobial Resistance |
| ARPEC | Antibiotic Resistance and Prescribing in European Children |
| AST | Aspartate Aminotransferase |
| AUC | Area under the time curve |
| AUIC | Area under the inhibitory curve |
| BID | Bi-daily (Twice daily) |
| BNF C | British National Formulary for Children |
| BPD | Broncho pulmonary dysplasia |
| CC | Central compartment |
| CFU | Colony Forming Units |
| CI | Confidence interval |
| Cmax | Concentration maximum |
| Cmin | Concentration minimum |
| CL | Clearance |
| COMET | Core Outcome Measures in Effectiveness Trials |
| CNS | Central Nervous System |
| CoNS | Coagulase-negative staphylococci |
| CRF | Case Report Form |
| CRP | C-Reactive Protein |
| CSF | Cerebrospinal Fluid |
| CSP | Centralised Service for NHS Permissions |
| CTA | Clinical Trial Authorisation |
| CTIMP | Clinical Trial Investigating a Medicinal Product |
| CV | Coefficients of variation |
| CWRES | Conditional weighted residuals |
| D | Day |
| DIC | Disseminated Intravascular Dissemination |
| DMC | Data Monitoring Committee |
| DNA | Deoxyribonucleic acid |
| DoH | Department of Health |
| DV | Dependent variable (observed concentrations) |
| EAB | Ethics Advisory Board |
| EARS-Net | European Antimicrobial Resistance Surveillance Network |
| ECDC | European Centre for Disease Prevention and Control |
| ECMO | Extra-Corporal Membrane Oxygenation |
| ECOFF | Epidemiological ‘wild type’ cut off |
| EMA | European Medicines Agency |
| EU | European Union |
| EUCAST | European Committee on Antimicrobial Susceptibility Testing |
| Eta (η) | Random effect |
| FDA | Food and Drug Administration (US) |
| FOCE | First order conditional estimation |
| FT | Foundation Trust |

| | |
|---|---|
| GA | Gestational age |
| GCP | Good Clinical Practice |
| GFR | Glomerular Filtration Rate |
| GN | Gram-negative |
| GNO | Gram-negative Organism |
| GNS | Gram-negative Sepsis |
| GOF | Goodness of fit |
| HPLC | High Performance Liquid Chromatography |
| H | Hour |
| HPA | Health Protection Agency |
| HR | Heart Rate |
| ICH GCP | International Conference on Harmonisation Good Clinical Practice |
| ICU | Intensive Care Unit |
| IDSMC | Independent Data Safety Monitoring Committee |
| IMP | Investigational Medicinal Product |
| IPRED | Individual predicted concentrations |
| IQR | Inter-Quartile Range |
| IUGR | Intrauterine growth retardation |
| IVH | Intraventricular Haemorrhage |
| IWRS | Absolute individual weighted residuals |
| Ka | Absorption rate constant |
| LADMER | Liberation, Absorption, Distribution, Metabolism, Elimination, and Response |
| LAMB | Liverpool Archive of MRI in Babies |
| LC-MS | Liquid Chromatography-Mass Spectrometry |
| LWH | Liverpool Women's NHS Foundation Trust |
| MALDI-TOF-MS | Matrix Assisted Laser Desorption Ionisation Time-Of-Flight Mass Spectrometry |
| MLST | Multilocus Sequence Typing |
| MHRA | Medicines and Health Care Products Regulatory Agency |
| MIC | Minimum Inhibitory Concentration |
| MPC | Mutant Prevention Concentration |
| MRC | Medical Research Council |
| MRI | Magnetic Resonance Imaging |
| MSOF | Multi system organ failure |
| NEC | Necrotising Enterocolitis |
| NHS FT | National Health Service Foundation Trust |
| NICU | Neonatal Intensive Care Unit |
| NONMEM® | Non Linear Mixed-effects Model |
| NPDE | Normalized prediction distribution errors |
| NRES | National Research Ethics Service |
| NTISS | Neonatal Therapeutic Intervention Scoring System |
| OAT3 | Organic Anion Transporter 3 |
| OFV | Objective function value |
| OR | Odds Ratio |
| OST | Optimal Sampling Strategy |
| PCR | Polymerase Chain Reaction |

| | |
|---|---|
| PD | Pharmacodynamics |
| PDA | Patent Ductus Arteriosus |
| PDCO | Paediatric Committee of the European Medicines Agency |
| PICU | Paediatric Intensive Care Unit |
| PIP | Paediatric Investigation Plan |
| PK | Pharmacokinetics |
| PMA | Post Menstrual Age |
| PMQR | Plasmid-Mediated Quinolone Resistance |
| PNA | Post-natal age |
| PRED | Population predicted concentrations |
| PUMA | Paediatric Use Marketing Authorisation |
| PTA | Probability of Target Attainment |
| Q | Inter-compartment clearance ($V_1$ and $V_2$) |
| QNR | Quinolone Resistance Gene |
| RDS | Respiratory Distress Syndrome |
| REC | Research Ethics Committee |
| RNA | Ribonucleic acid |
| RSI | Reference Safety Information |
| ROC | Receiver Operating Characteristic |
| RR | Relative Risk |
| SAE | Serious Adverse Event |
| SAGM | Saline, Adenine, Glucose and Mannitol |
| SIRS | Systemic Inflammatory Response Syndrome |
| SmPC | Summary of Product Characteristics |
| SOP | Standard Operating Procedure |
| SI | Statutory Instrument |
| SSI | Site Specific Information |
| SUSAR | Suspected Unexpected Serious Adverse Reaction |
| SVD | Source Verification Data |
| T (number) | Time point |
| TA-MC | Transfusion Associated-Microchimerism |
| t (tau) | Dosage interval (time) |
| TBW | Total Body Water |
| TDM | Therapeutic Drug Monitoring |
| TID | Three times daily |
| TINN | Treat Infection in Neonates Consortium |
| UNICEF | United Nations Children's Fund |
| Vd | Volume of Distribution |
| V1 | Central volume of distribution |
| V2 | Peripheral volume of distribution |
| VLBW | Very Low Birth Weight |
| WHO | World Health Organisation |
| WSCV | Within Subject Coefficient of Variation |
| WT | Wild Type |
| θ | Theta |

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# Chapter 1 Introduction

## 1.1 Neonatal Infection

Every year, an estimated four million babies die in the first-four weeks of life [1]. More than 80% of all newborn deaths result from three preventable and treatable conditions: infection, complications due to prematurity and intrapartum-related deaths (including birth asphyxia) [2]. In the UK the neonatal mortality rate has reduced over the last 30 years from 7.7 per 1000 live births (1980) to 2.9 per 1000 live births in 2010 [3]. The average global neonatal mortality rate is 20 deaths per 1000 live births but rises in developing countries to 30 per 1000 live births [2]. Inequalities in health care are evident but also suggest that improvements in neonatal care can improve mortality.

The United Nation's Millennium Development Goals aimed to reduce child mortality by two-thirds, between 1990 and 2015 [4]. Mortality for the sub-group of neonates is reducing at a slower rate than the broad group of under-five-year-olds. Neonates accounts for 44% of mortality in children under five years old [4] Figure 1-1. Infectious diseases account for more than four million deaths a year in children (58%) [4]. An action plan to end preventable deaths 'Every Newborn' by WHO and UNICEF in 2014 set a target to reduce the neonatal mortality rate to ten or fewer newborn deaths per 1000 live births by 2035 [2]. Infection is an ever-present threat in this highly vulnerable population and these recent reports suggest an increasing need for effective treatment. As neonatal mortality improves, the surviving babies are at increased risk of morbidity increasing the burden of infection [5].

There remains considerable uncertainty about the optimal way to use antibiotics in neonates, particularly the optimal regimens to improve clinical outcomes and prevent the emergence of resistant organisms. Optimising the use of existing antibiotics is important particularly due to the decline in new antibiotics [6].

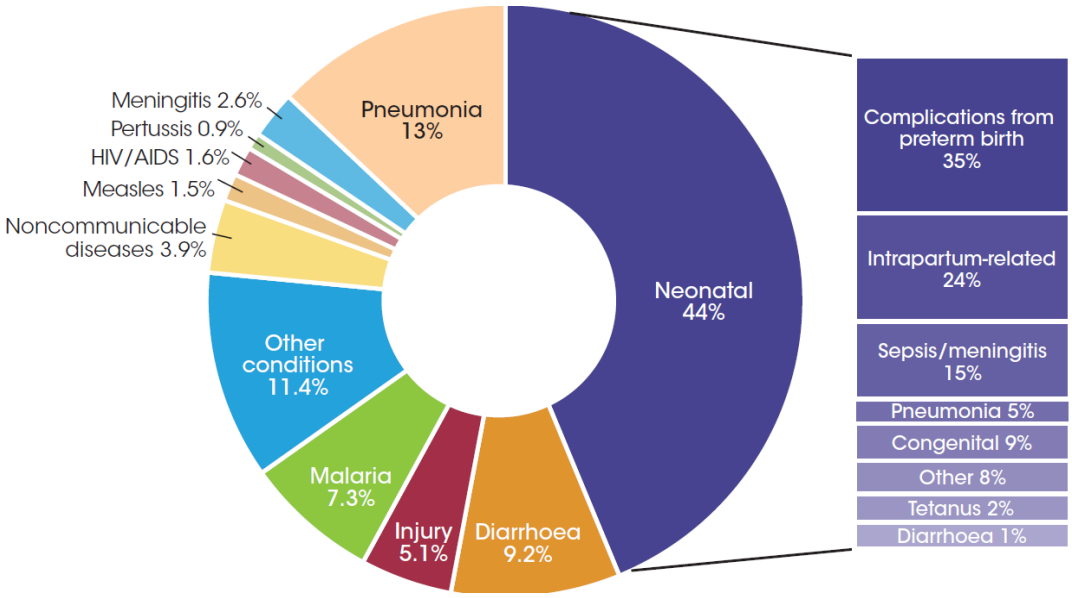



Figure 1-1 Causes of deaths in children under 5 years age 2012

WHO Global Health Observatory Data 2014 (accessed 2015)
**http://apps.who.int/gho/data/node.wrapper.MORT-**

Fourteen classes of antibiotic were developed for human use between 1935 and 1968 but subsequently only five have been introduced to clinical practice

Figure 1-2 http://antibiotic-action.com/aa-resources/ (accessed August 2013).

Equally, there is a growing gap between the availability of new antibiotics and the increasing incidence of infections caused by multi-resistant bacteria [6].

In Europe, the human and economic burden on healthcare of antibiotic-resistant Gram-negative organisms is predicted to outweigh that of MRSA especially for E.coli [6]. To suppress the emergence of resistant organisms it is important to identify optimal regimens with pharmacodynamic modelling that result in effective clinical and microbiological outcomes [6, 7]. The impact of infection can be reduced by antimicrobial stewardship [6]. However the treatment of sepsis does not always protect infants from subsequent long-term neurodevelopmental impairment. The best strategy is to prevent rather than to treat late onset sepsis (LOS) [8].

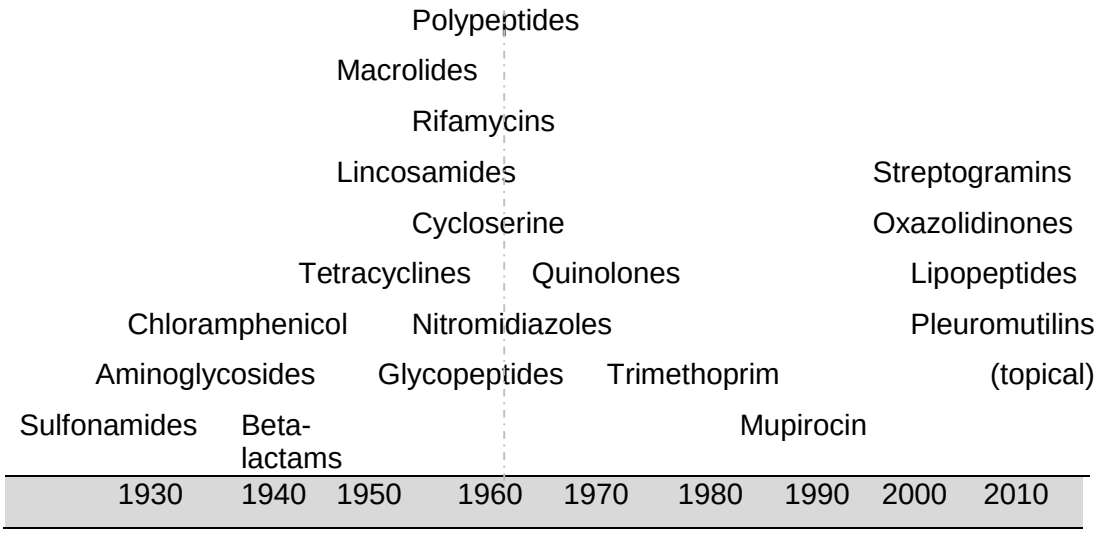



Figure 1-2 Discovery of new classes of antibiotics

Adapted from ECDC Report The bacterial challenge : time to react [6]

Three main goals of antimicrobial therapy are relevant to neonatal infection [7]. Firstly, the antimicrobial agent(s) must be initiated as soon as possible after the onset of sepsis [9]. The protocol in the neonatal unit at Liverpool Women's NHS Foundation Trust (FT) requires administration within 30 minutes of the decision to treat suspected sepsis. Secondly, the antimicrobial spectrum of the agent should be broad enough to cover the potential causative microorganisms [10]. Therefore, empiric antimicrobial therapy is often administered before the causative pathogen is known [11, 12]. Finally, an appropriate antimicrobial dosing regimens is required to maximize microbial killing, minimize the development of multidrug antimicrobial resistance, and avoid concentration-related adverse drug reactions [13, 14]. The success of each step is dependent on pharmacokinetic and pharmacodynamic (PK-PD) relationships specific to the neonatal population.

### 1.1.1 The Clinical Impact

Suspected sepsis is one of the most common diagnoses made in the neonatal intensive care unit. It is difficult to diagnose as the signs of sepsis are nonspecific, and inflammatory syndromes of non-infectious origin often mimic those of neonatal sepsis [15, 16]. In most cases a diagnosis is based on clinical suspicion which has a reasonably reliable a positive predictive value of approximately 70% [17]. Infection that occurs in the days after birth is defined as early onset. This is thought to be due to organisms acquired before, or during birth including those that ascend from the birth canal. Late onset is more likely to be nosocomially acquired [16, 18]. The cut-off for early vs. late neonatal infection has not been defined and definitions vary between 48 hours and 6 days [11]. One-third of the late onset neonatal infections in developed countries are associated with Gram-negative organisms [18, 19].

Since the clinical presentation of sepsis is non-specific, many neonates are empirically treated. Few neonates treated for sepsis have culture proven sepsis, although the incidence increases from 2% for early onset infection to 25% for late onset infection [18]. Stoll *et al.* reported half of their cohort of neonates with suspected sepsis received antibiotics but only 21% had a positive blood culture [18]. Blood cultures may be falsely negative as the sensitivity is dependent on the bacterial burden in the blood and obtaining a sufficient blood volume [20, 21].

To make the diagnosis of neonatal sepsis even more challenging, bacteraemia may occur in the absence of clinical signs. The subtlety of these signs also makes it difficult to determine when to discontinue antimicrobial therapy [16]. Thus diagnostic uncertainty results in the over-use of antibiotics and “spiralling empiricism” [22]. Prolonged empiric antibiotic therapy in premature babies has been associated with the combination of late onset sepsis, necrotising enterocolitis (NEC), and death (OR, 2.66, 95% CI 1.12-6.3) [23]. Rapid identification of causative organisms may reduce the use of broad spectrum antibiotics but is not readily available at present.

Rates of neonatal infection are inversely related to birth weight and gestational age [18]. A higher proportion of very premature neonates (36.3%) GA <28 weeks had at least one episode of late onset sepsis when compared with 29.6% of moderately preterm (GA 29–32 weeks) and 16.5% term infants [24]. Gram-negative neonatal sepsis is a significant problem especially among VLBW infants [25, 26].

The Expert Meeting on Neonatal and Paediatric Sepsis European Medicines Agency 2010 [27] defined neonatal sepsis as the presence of at least two clinical symptoms and at least two laboratory signs in the presence of or as a result of suspected or proven infection (positive culture, microscopy or polymerase chain reaction) Table 1-1. Other definitions include 'a clinical syndrome characterised by systemic signs of infection accompanied by bacteraemia in the first month of life' [28]. Sepsis in children was defined by the International Consensus Conference on Paediatric Sepsis and Organ Dysfunction (2002) [29] as "a systemic inflammatory response syndrome (SIRS) in the presence of, or as a result of, suspected or proven infection" requiring a minimum of two symptoms one of which must be either:

- an abnormal temperature and/or leukocyte count
- or both tachycardia and altered respiratory rate

They excluded neonates from their definition of sepsis in children because of the complexities of diagnosis [29].

As the survival of critically ill neonates increases, the period of hospitalisation is prolonged with an inevitable increase in infection and associated morbidity. A study monitoring the outcome of babies with septic shock found that only 28% were alive and considered normal at 18 months follow up [30]. Post-natal infection is an important risk factor for subsequent morbidity including neurodevelopmental abnormalities [31]. Recurrent postnatal infection may be detrimental to early brain development. The brains of premature and VLBW infants in particular may be affected directly (e.g. meningitis) or as a result of inflammatory injury, [32-34]. Inflammation is

associated with progressive white matter injury in premature infants that has been attributed to the susceptibility of oligodendrocyte precursors to inflammation, hypoxia, and ischemia [19]. Neurodevelopmental sequelae (e.g. cognitive and psychomotor impairment, cerebral palsy, and visual impairment) are independent of the inciting pathogen [34].

Mortality caused by Gram-negative infection is significantly higher than that of Gram-positive infections at all ages of sepsis onset [25]. Mortality from Gram-negative late onset sepsis is approximately 36% which is higher than estimated for other infections (18%) and the overall mortality of VLBW babies without sepsis (7%) [18]. Fulminant late-onset sepsis (i.e. lethal within 48 hours) is more likely to be caused by Gram-negative organisms [35, 36]. There are important differences in mortality as a function of the invading pathogen. Gram-negative bacteria produce endotoxin that incites a systemic inflammatory response [37]. Higher mortality rates are associated with *Pseudomonas* (74.4%) compared with other members of *Enterobacteriaceae* (e.g. *E. coli* (34%), *Serratia marcesens* (35.9%), *Klebsiella* spp. (22.6%) and *Enterobacter* spp. (26.8%) [34].

Table 1-1 Clinical results and signs of sepsis in neonates (<44 weeks PMA)

| **Modified Body Temperature** |
|---|
| Body temperature >38.5 $^{0}$C or <36 $^{O}$C |
| AND/OR |
| Temperature instability |
| **Cardiovascular Instability** |
| **Bradycardia** (mean HR <10$^{th}$ percentile for age in the absence of external vagal stimulus, beta-blocker or congenital heart disease |
| **OR** |
| otherwise unexplained persistent depression over a 0.5 h time period) |
| **OR** |
| **Tachycardia** (mean HR >2 SD above normal for age in the absence of external stimulus chronic drugs and painful stimuli or otherwise unexplained persistent elevation over a 0.5 h to 4 h time period |
| **AND/OR** |
| Rhythm instability |
| Reduced urine output (<1mL/kg/h) |
| Hypotension (mean arterial pressure <5$^{th}$ percentile for age) |
| Mottled skin |
| Impaired peripheral perfusion |
| **Respiratory Instability** |
| Apnoea episodes OR |
| Tachypnoea episodes |
| Mean respiratory rate > 2 SD above normal for age |
| Or increased oxygen requirements |
| Or requirements for ventilation support |
| **Skin and subcutaneous lesions** |
| Petechial rash / sclerema |

| **Haematological and Biochemical** | |
|---|---|
| White Blood Cells | <4000 x10$^{9}$ cells /L |
| or | >20 000 x 10$^{9}$ cells/L |
| Immature to total neutrophil ratio (IT) >0.2 | |
| Platelet count | >100000 x10$^{9}$ cells/L |
| C Reactive Protein | >15mg/L OR |
| Procalcitonin | ≥ 2$^{1}$ ng/mL |
| Glucose intolerance confirmed at least 2 times | |
| Hyperglycaemia | >10 mMol/L |
| Hypoglycaemia* | <2.5 mMol/L |
| **Metabolic acidosis** | |
| Base Excess BE | < -10 mEq/L |
| Or | |
| Serum Lactate | > 2 mMol/L |
| **Gastrointestinal** | |
| Feeding intolerance | |
| Poor sucking | |
| Abdominal distension | |
| **Non-specific** | |
| Irritability | |
| Lethargy | |
| Hypotonia | |

**when receiving age specific normal range of glucose*

*Adapted from EMA report on the Expert meeting of Neonatal and Paediatric Sepsis 2010 [27]*

### 1.1.2 Microbiology

The range of organisms causing late onset sepsis includes Gram-positive or negative bacteria and fungi [38]. Gram-negative bacteria account for one third of late onset sepsis primarily *Enterobacteriaceae* [18, 34]. Gram-negative organisms belong to two broad groups. *Enterobacteriaceae* known as lactose fermenting coliforms includes e.g. *E.coli, Enterobacter* spp., *Hafnia*, *Klebsiella* spp., *Serratia* and *Citrobacter.* These are all late fermenters. The other group includes non-lactose fermenting e.g. *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Acinetobacter* spp., *Stenotrophomonas* and *Haemophilus influenzae* [25]. Currently, the most common Gram-negative organisms isolated in cases of neonatal sepsis are *E.coli, Klebsiella* spp. and *Pseudomonas* spp. [11, 18, 25, 26, 39, 40] Figure 1-3.

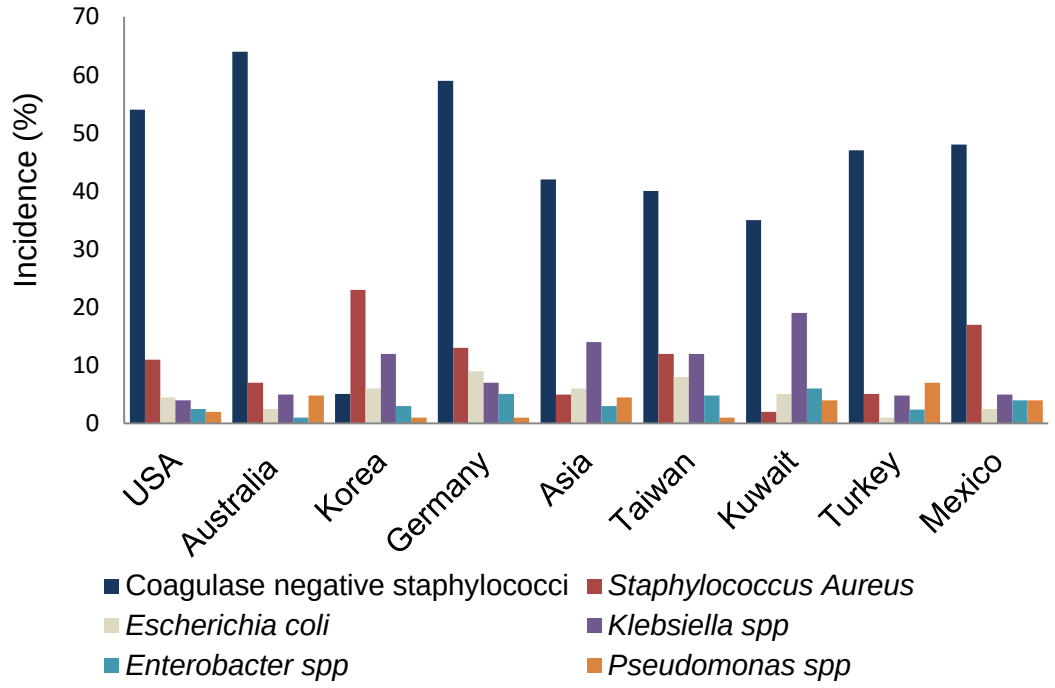



Figure 1-3 Major causative pathogens of neonatal late-onset sepsis globally

Adapted from Dong et al (BMJ Publishing Group) [11].

A recent survey in the UK, found bacteria isolated from neonatal blood cultures between 2006 and 2008 were mainly Gram-positive (80%) of which the main organism was Coagulase-negative staphylococci (CoNS) (45.1%); the main Gram-

negative organisms (19.3%) including *Enterobacteriaceae* (9%), *E.coli* (6.8%) and *Pseudomonas* spp. (1.9%) [12]. CoNS have emerged as the predominant pathogens of late onset sepsis, accounting for 53.2%–77.9% of late onset sepsis in industrialised countries and 35.5%–47.4% in some developing regions [11]. The ARPEC survey in European found the most common Gram-negative isolates from infants under one year were Escherichia coli 27.9%, Klebsiella pneumoniae 13.2 and Pseudomonas aeruginosa 6.1[41].

Neonates are colonised by three main types of bacteria: 1) beneficial (lactobacilli and bifidobacteria), 2) potentially pathogenic (*Enterobacter* spp., E*nterococci* and *bacteroides*) 3) pathogenic (*Clostridia, Staphylococci, Klebsiella* spp. and *Pseudomonas* spp.) [42]. Colonisation of the gut in intensive care is altered by delayed feeding and antibiotic administration [42, 43]. Meconium is not sterile and the neonatal gut may be colonised with potentially pathogenic Gram-negative bacteria including *E.coli* and *Pseudomonas* spp. within two days of birth [43-45].

Antibiotics may supress the growth of susceptible organisms leaving other bacteria to dominate then translocate across the gut leading to sepsis with significant morbidity and mortality [46, 47]. The use of broad spectrum antibiotics causes a less diverse gut flora that is associated with late onset sepsis [43]. This is a particular concern for inpatients. Pathogens acquired within a hospital are more likely to have previous antibiotic exposure therefore at greater risk of harbouring established resistance mechanisms [48].

#### 1.1.2.1 Mechanism of resistance

Fluoroquinolones are novel among antimicrobial agents as they directly inhibit DNA synthesis. Ciprofloxacin's pharmacological action is that it binds to the topoisomerases; these are essential enzymes for replication of bacteria DNA, they target two of the four topoisomerases enzymes DNA *gyr*ase II and IV [49, 50]. Both are large complex enzymes composed of two pairs of subunits *gyr*A and *gyr*B that

work together in the replication, transcription, recombination and repair of DNA [51]. The topoisomerase structure has two subunits coded by parC and parE genes [52]. Until recently the mutations that prevented it from binding were the most important mechanisms of ciprofloxacin resistance [50, 53]. In Gram-negative bacteria *gyr*ase II is more susceptible to inhibition by quinolones whereas Gram-positive bacteria topoisomerase IV is the target and their *gyr*ase is intrinsically less susceptible. Consequently, resistant mutations occur first in *gyr*A in Gram-negative bacteria and in parC in Gram-positive bacteria [51]. A single mutation in *gyr*A will produce only modest increments of resistance so bacteria would still be clinically susceptible, a second mutation or mutation in parC would be required for clinical resistance to be reached [51].

Isolates with decreased ciprofloxacin susceptibility, emerged in the 1990s and usually had single point mutations in the quinolone resistance determining region (QRDR) of the *gyrA* gene [54, 55]. One mutation may exhibit elevated MICs which are difficult to distinguish from the wild-type MIC distributions [56]. Multiple mutations generally lead to resistance to all active substances within the class [52]. However, in some species that are inherently less susceptible to fluoroquinolones it has been suggested that only one mutation is needed for clinically relevant resistance to occur [57]. *In vitro* investigations have shown that resistance to ciprofloxacin is commonly acquired by a stepwise process by target site mutations [53, 56]. Cross-resistance between fluoroquinolones may occur when the mechanism of resistance is due to mutations in bacterial *gyr*ases.

Impermeability and/or drug efflux pump mechanisms that are reverse transport systems pumping the drug out of the cell may have a variable effect on susceptibility to fluoroquinolones. This depends on the physicochemical properties of the various drugs within the class and the affinity of transport systems for each drug. [53]. Low concentration resistance mechanisms include increased activity of efflux pumps [58]

where antibiotics enter the bacterial cell via a porin channel but are then ejected [50, 57, 59]. Efflux pumps have been found in *Stenotrophomonas maltophilia* (intrinsically resistant), *P.aeruginosa* and *Acinetobacter baumannii* [60, 61]. Mechanisms of resistance to quinolones include plasmids that protect cells from the lethal effects of quinolones [49]. Fluoroquinolones are small allowing them to cross the outer membrane through porin proteins therefore resistance is associated with a reduction in porins [62].

The mechanisms of ciprofloxacin resistance identified a quinolone resistance protein, this plasmid-mediated quinolone resistance gene named 'Qnr' is known as plasmid-mediated quinolone resistance (PMQR) that is capable of modifying ciprofloxacin to reduce its activity [51] [50]. The genetic transfer via a plasmid-encoding quinolone (Qnr A) resistance pentapeptide protein is capable of protecting the DNA *gyr*ase from quinolones and inactivating enzymes [56, 63]. Horizontal gene transfer is a concern as DNA mutations can be transferred to either individual bacteria or different genus or species, whereas vertical transfer limits resistance to the same species. Martinez et al found a *Klebsiella pneumoniae* isolate transferred low level quinolone resistance to *E coli* and other Gram-negative bacteria [64]. Since then this has been identified worldwide in the US, Europe and Asia [50]. Another concern is that the basal level of resistance in wild type strains was low, but plasmid mediated resistance allows the bacterial population to reach a concentration at which secondary mutations to high resistance can occur. Ultimately, this may hasten the widespread loss of susceptibility of fluoroquinolones to many Gram-negative bacteria [64]. Robicsek et al reported that the mechanisms and spread of these plasmids into apparently susceptible organisms have led to four to eight fold higher MICs for ciprofloxacin [50]. Robicsek *et al.* advise a more cautious approach to quinolone use is required and raises concerns about the appropriateness of current quinolone breakpoints [50, 65].

Subtle changes in MIC may predispose to sub–clinical resistance and possibly lead to a reduced effectiveness of fluoroquinolones over time [52, 66-68]. Higher drug concentrations may provide some protection against resistance; lower rates of fluoroquinolone resistance *in vitro* and *in vivo* have been found with higher $AUC_{24}$/MIC ranges [69]. A change in MIC over the period of treatment with an antibiotic may indicate that ciprofloxacin resistance has occurred. Subtle changes in the MIC are more easily detected by testing against the lower class quinolone nalidixic acid [63]. Time to kill studies have shown that when the dose was double the MIC or higher than the mutant prevention concentration (MPC), resistant mutants did not arise [63].

#### 1.1.2.2 Prevalence of Gram-negative resistance

Antimicrobial resistance surveillance in Europe 2014 restated that the situation for gram-negative bacteria is especially worrying but varies throughout Europe influenced by prescribing practices. Within the UK fluoroquinolone resistance is lower than the European mean 22.4% (95% CI 22 to 24) and has decreased between 2011 and 2014 from 17.5% (17-19) 16.8 % (16-18) [70] Table 1-2. In neonatal late onset sepsis Gram-negative bacteraemia reported to the UK Health Protection Agency (HPA) between 2006 and 2008 found higher resistance to cefotaxime monotherapy (23%) compared to combination therapy with flucloxacillin and gentamicin (4%), amoxicillin and cefotaxime (15%) or amoxicillin and gentamicin (7%)[71]. As few as 26 Gram negative bacteraemia isolates are reported per month for neonates as reporting to the HPA is not compulsory neonatal data for clinical trials or clinical breakpoints is limited [71]. A prevalence report by Antibiotic Resistance and Prescribing in European Children (ARPEC) found Gram-negative resistance in infants less than one year of age was less than older children and adults reported by European Antimicrobial Resistance Surveillance Network (EARS-Net) between 2011 and 2012 [41]. In particular, aminoglycoside resistance is increasingly alarming as this is considerably higher in children older than one year age compared to younger neonates, infants or adults

Table 1-3. There is a lower rate of resistance in neonatal intensive care but the authors suggest the burden of resistance could still be highest in this group due to the higher incidence of blood stream infections [41]. Third generation cephalosporins in *Klebsiella pneumoniae* and *Escherichia coli* increased significantly between 2011 and 2014 in Europe. This was increasingly seen in combination with fluoroquinolone and aminoglycoside resistance from 16.7 % in 2011 to 19.6 % in 2014 [70].

Table 1-2 Percentage of Resistance comparing the UK with Europe
(adapted from ECDC report 2014 [70])

| **Organism** | **Antimicrobial** | **Resistance %** | | | |
|---|---|---|---|---|---|
| | | **UK** | | **Europe 2014** *Population weighted mean except for median* (95% CI) | |
| | | **2011** | **2014** | | |
| *Acinetobacter* | Aminopenicillin | - | - | - | - |
| | Aminogylcoside | - | 10 | 38 * | (22-56) |
| | Carbapenem | 1 | 2 | 26 * | (13-44) |
| | 3rd Generation Cephalosporins | - | - | - | - |
| | Fluoroquinolone | | 11 | 41 * | (25-59) |
| *Escherichia coli* | Aminopenicillin | 63 | 63 | 57 | (56-58) |
| | Aminogylcoside | 8 | 9 | 10 | (9-11) |
| | Carbapenem | <1 | <1 | <1 | <1 |
| | 3rd Generation Cephalosporins | 10 | 10 | 12 | (11-13) |
| | Fluoroquinolone | 18 | 17 | 22 | (21-24) |
| *Klebsiella* | Aminopenicillin | - | - | - | - |
| | Aminogylcoside | 4 | 6 | 23 | (21-25) |
| | Carbapenem | <1 | 1 | 7.3 | (6-9) |
| | 3rd Generation Cephalosporins | 5 | 9 | 28 | (25-30) |
| | Fluoroquinolone | | 8 | 27 | (26-30) |
| *Pseudomonas aeruginosa* | Piperacillin/ Tazobactam | 5 | 5 | 17 | (14-21) |
| | Aminogylcoside | 3 | 2 | 14.8 | (12-18) |
| | Carbapenem | 6 | 6 | 18.3 | (15-21) |
| | 3rd Generation Cephalosporins (Ceftazidime) | 5 | 5 | 13 | (10-16) |
| | Fluoroquinolone | | 5 | 19 | (16-22) |

Table 1-3 Antimicrobial resistance of Gram-negative pathogens comparing adults and infants between 2011 and 2012
(adapted from ARPEC and EARS – NET data [41]

| **Gram-negative Pathogen** | **Antibiotic** | **Resistance %** | | |
|---|---|---|---|---|
| | | **Adult** | **Children >1 year age (95% CI)** | **Infants <1 year age (95% CI)** |
| *Escherichia coli* | Aminoglycosides | 11.3 | **16.7 (9.9-23.4)** | 13.5 (9.0 -18) |
| | Carbapenems | 0.1 | 1.7 (0.2-5.9) | 0 (0-1.7) |
| | 3rd Generation cephalosporins | 11.9 | 17.0 (10.2-24.0) | 10.7 (6.6 -14.8) |
| | Fluoroquinolones | 23 | 22.5 (14.9-30.1) | 8.5 ( 4.8 -12.2) |
| *Klebsiella pneumoniae* | Aminoglycosides | 27.6 | **41.2 (29.0 – 54.4)** | 26.2 (18.1 - 35.6) |
| | Carbapenems | 13.5 | 14.4 (6.7-25.4) | 1.9 (0.2 - 6.7) |
| | 3rd Generation cephalosporins | 31.6 | 37.1 (25.2-50.3) | 29.9 (21.4 - 39.5) |
| | Fluoroquinolones | 30.7 | 35.5 (23.7-48.7) | 7.5 (3.3 - 14.3) |
| *Pseudomonas aeruginosa* | Aminoglycosides | 19.3 | **35.4 (25.0-47.0)** | 14.3 (5.9 -27.2) |
| | Carbapenems | 20.5 | 36.7 (26.1-48.3) | 26.1 (14.3 - 41.1) |
| | Ceftazidime | 14.8 | 34.3 (23.8-45.7) | 12.2 (4.6 - 24.8 |
| | Fluoroquinolones | 23.1 | 27.8 (18.3-39.1) | 16.2 (7.3 - 29.7) |

### 1.1.3 Neonatal Pharmacology

Variability in drug response among patients is multifactorial, including environmental, genetic and disease determinants that affect the disposition of a given drug. The interplay of these factors determines the profile of the plasma concentration over time and pharmacological effect at the site of infection [72].

To guide drug therapy the regulatory process ensures that a medicinal product undergoes extensive studies to ensure that it is safe, manufactured to high quality standards to be authorised specifically for use in the target population [73]. Unmonitored off label use of medicines in children extrapolated from adult data has resulted in significant morbidity and mortality; due to toxicity and under-dosage which results in ineffective treatment. The lack of data required for a Marketing Authorisation with the European Medicines Agency (EMA) prevents children from benefiting from therapeutic advances [73, 74]. To obtain a Marketing Authorisation for a drug for children the EMA require a Paediatric Investigation Plan (PIP) that outlines drug development specific to the population that may include pharmacokinetic, pharmacodynamic and pharmacogenomic studies[73]:

- **Pharmacokinetics (PK)** - how the body handles the drug. The changes in drug concentration in the different body fluids and tissues in the dynamic system of liberation, absorption, distribution, body storage binding metabolism and excretion [75].
- **Pharmacodynamics (PD)** - the *action* of the drug in the body, i.e. what the drug does to the body [76].
- **Pharmacogenetics** – the individual variation in DNA sequence related to drug absorption and distribution (pharmacokinetics) or drug action (pharmacodynamics) [75]

The application of these general principles of antibiotic development to neonates faces many challenges. At present there are no authorised medicinal products for the treatment of sepsis in neonates or children [27]. Some antibiotics are authorised for infections in specific sites. As recently as 1999 most neonates (90%) surveyed had

received at least one off label drug during their admission [77]. In Europe the paediatric drug development climate has changed dramatically. The EU Paediatric Regulations (2006) recognise that drug development specific to the population is required and aim to ensure medicines are authorised specifically for children [73].

It is widely accepted that the developmental changes in the spectrum of premature and term neonates influence the response to therapy [78-80]. The absorption, distribution, metabolism and elimination (ADME) of drugs by neonates is different not only to adults but to sub-groups of children. Previous attempts to scale adult dosages to children have failed as age associated changes in body composition and organ function are not linear per kilogram [78, 81]. As a result of this the EMA require neonatal trials to be stratified representing the changes for each four week period of development [79, 80].

### 1.1.4 Pathophysiology of Critical Illness

In critical illness many factors influence the PK/PD of drugs including changes in physiology due to the clinical condition and the disease influence on organ function Figure 1-4. The key influence of ciprofloxacin in neonates is yet to be determined but the following factors are considered and included in the case report form as potential covariates for the PK clinical trial. Physiology may be altered within hours by systemic inflammatory response, multi system organ failure, drug interactions that occur during poly-therapy, instability of renal function or interventions such as extracorporeal circulation or dialysis [82]. This may result in decreased attainment of PD targets or development of toxicity. In addition, the effect of other medicines that are administered simultaneously may affect the PK parameters of the investigational medicine.

Clinical interventions that provide organ support including ventilation ECMO /cardiopulmonary bypass dialysis/haemofiltration may influence drug disposition [83]. Positive pressure ventilation has been shown to influence the clearance of vancomycin in neonates perhaps via decreased atrial natriuretic peptide and

increased anti-diuretic hormone production [84]. This may be attributable to right atrial pressure receptors that are influenced by altered thoracic pressures during positive pressure ventilation. Factors specific to renal replacement therapy may even include differences in the type of haemofiltration filter and ultra-filtration rate [85].

Ongoing evaluation of illness severity allows timely adjustment of antibacterial dosing to minimise antibacterial resistance Figure 1-4 [86]. Antimicrobial Resistance (AMR) has been shown to increase with suboptimal drug exposure [87]. Regular monitoring of drug concentrations may be required for drugs with a narrow therapeutic index.

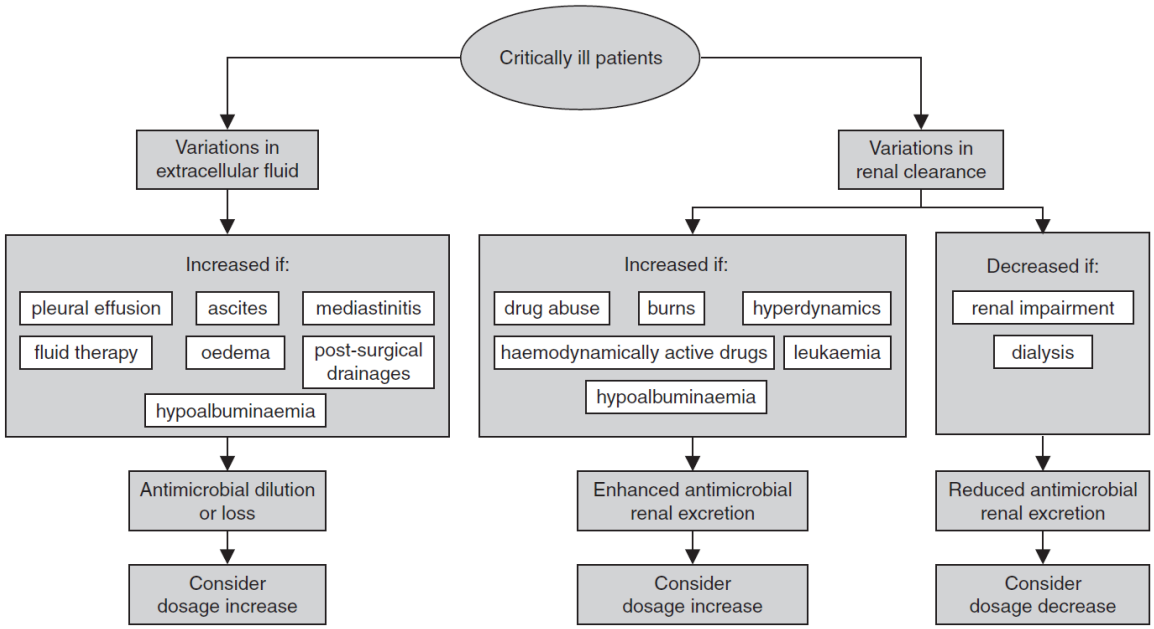



Figure 1-4 Pathophysiology during critical illness and PK effects
Reproduced from Roberts et al 2006 Springer Publications [86]

Hypoalbuminaemia occurs frequently in critical illness due to a leaky endothelium, fluid overload, malnutrition and fluid extravasation. The effect of hypoalbuminaemia is that less of the drug binds to albumin; the increase in the free fraction of drugs may increase the volume of distribution but also facilitates elimination (see 1.1.5.2.2) [88, 89]. The inflammatory response to sepsis leads to capillary leak. Fleck *et al.* found a 200% increase in trans-capillary leak from intravascular to extravascular space during the first 2 days of admission in critically ill adults with a mean serum albumin of 25g/L

[90]. Severe hypoalbuminaemia (22 ± 6.1 g/L) resulted in a substantial increase in the volume of distribution of ceftriaxone and almost doubled drug clearance (41.3 ± 11.7 vs 19.8 ± 2.5 mL/min) compared to healthy volunteers with normal renal function [91]. A significant covariate for amikacin interindividual variability was hypoalbuminaemia that increased the Vd to the extent that a loading dose 1.6 fold higher was required [92]. A low albumin may result in the unbound drug being distributed into a larger extravascular compartment faster resulting in low concentrations in the distribution phase [89]. Conversely, when protein concentrations are high the drug concentration retained in the plasma space will be greater [75]. Even if clearance remains constant, the half-life ($T_{1/2}$) may increase with implications on the frequency of administration of the drug [93]. As capillary leak resolves, the half-life may then increase when the drug is redistributed from the tissues back into the blood [89].

In summary clearance of ciprofloxacin is primarily renal and the effect of sepsis or immaturity on renal function are expected to be significant. Ciprofloxacin it is only moderately bound to protein 20-40% therefore changes in albumin may have less effect on concentrations than other drugs [53]. As ciprofloxacin is lipophilic concentrations are less likely to respond to the fluctuating effect of changes in fluid volume due to capillary leak or fluid resuscitation throughout the sepsis episode. Similarly, these factors are associated with developmental changes discussed in more details in the ontogeny section.

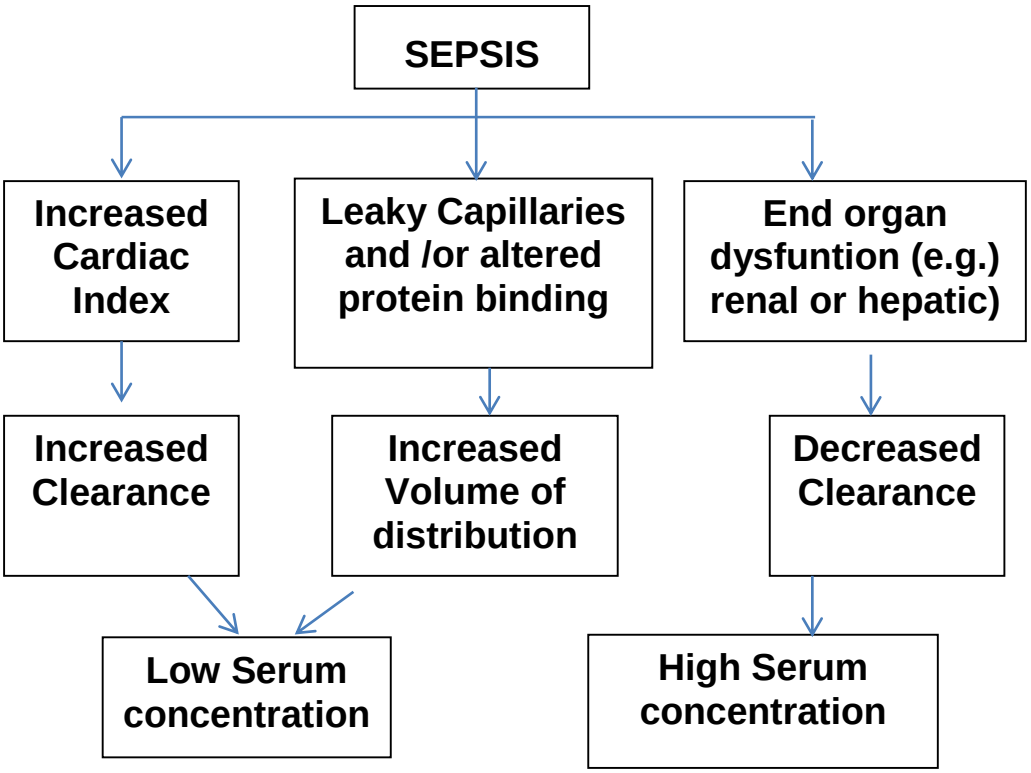



Figure 1-5 Pathophysiological changes during sepsis affecting concentration

This illustrates pathophysiological changes during sepsis that affect distribution and elimination of antimicrobials. Reproduced from Pea et al 2005 Springer Publications [88]

### 1.1.5 Ontogeny

Ontogeny is the study of the development of an organism [94]. Changes in body composition, and organs influence drug disposition [78]. These developmental changes influence metabolic capacity, distribution of total body water, extracellular water, body fat and renal function illustrated by Figure 1-5. The Liberation, Absorption, Distribution, Metabolism, Elimination and Response referred to as "LADMER" describes the relationship between a dose and the ultimate effect [75].

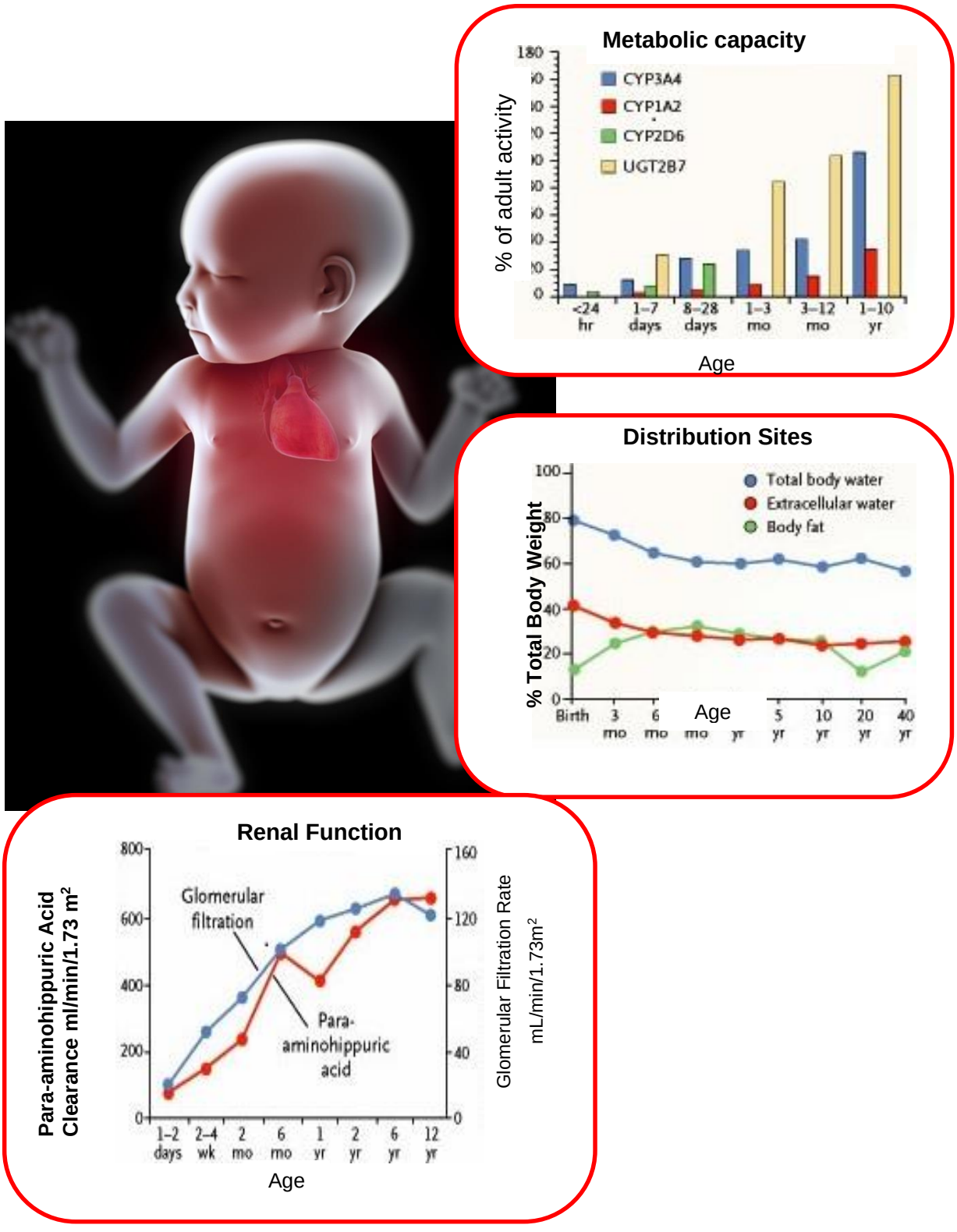



Figure 1-6 Developmental changes and physiologic factors influencing the disposition of intravenous drugs

Adapted from: Factors that Influence Drug Disposition in Infants, Children, and Adolescents. Reproduced with permission from Massachusetts Medical Society [78].
Image of infant reproduced with permission from Science Picture.Co

#### 1.1.5.1 Absorption

In neonatal sepsis antibiotics are administered intravenously [75]. Due to the high mortality and morbidity associated with neonatal sepsis other routes of drug administration are not reliable. There is a risk of reduced bioavailability caused by delayed gastric emptying and possible differences in intramuscular blood flow following intramuscular administration [79, 93].

#### 1.1.5.2 Distribution

The distribution of drugs in neonates differs to older children depending on their developmental stage Figure 1-7. The anatomical distribution of the drug is influenced by the proportion of body water in the physiological spaces, the high surface area to weight ratio, and protein or fat content [95]. Also, there are changes in haemodynamics, cardiac output, regional blood flow and membrane permeability that influence the distribution of a drug [80, 84].

During the first year of life there is a sharp decrease in the total body water (TBW) that varies inversely with the amount of fat tissue in the body. The TBW constitutes 85% of the body weight in the preterm neonate, 78.4 % in term neonates reducing to approximately 60% at five months [96]. The proportion of extracellular water decreases during the first year of life from 40% in the newborn to 25% at one year; intracellular water increases during the first three months from 34% to 43% [96]. In the 3-month foetus the total body water is 92% of which extracellular fluid is 65% and intracellular 25% [97]. Neonates, young infants and children have a higher relative extracellular and total-body water spaces and higher water to fat ratio. The clinical relevance of this gradual reduction in body water in extracellular compartments with age is that higher doses of drugs per kilogram are required to achieve comparable plasma and tissue concentrations for drugs that distribute into the extracellular fluid [98]. The relatively larger extracellular and total body water spaces, and adipose stores in babies results in lower plasma concentrations of drugs when administered

in a weight-based fashion [78]. Conversely, when calculated as a percentage of litres per square meter TBW increases from 12.38 L/m2 to 12.9 at 12 months [96]. This distribution may influence the selection of allometric methods of dosing based on weight or surface area [99].

The volume of distribution (Vd) is a reflection of the drugs concentration in the extracellular (particularly intravascular) compartment [96]. A change in body water can result in a higher volume of distribution of soluble drugs in childhood with a longer half-life than in the adult [93]. The apparent volume of distribution (Vd) describes the relationship between the amount of drug in the body and it's plasma concentration [100]. V is simply a theoretical volume representing the total amount of drug in the body store required to achieve the same concentration in plasma when distributed throughout the body [100].

$$V = D/C_o$$

$$\text{Volume of distribution (V)} = \frac{\text{Dose (D) administered (total drug in the body)}}{\text{Initial plasma concentration } (C_o) \text{ of the drug}}$$

The higher total body water of a newborn baby, results in a higher volume of distribution compared with an adult and alters the pharmacokinetics Figure 1-7 [93]. An increase in blood volume, fluid retention due to renal failure or an altered fraction of unbound drug in the blood may result in an increased volume of distribution [84].

Table 1-4 Developmental aspects of fluid compartments as % of body weight

| **Post Menstrual Age** | **% Total Body Water** | **% Extra Cellular Fluid** | **% Intracellular Fluid** |
|---|---|---|---|
| <3 month fetus | 92 | 65 | 25 |
| Term | 75 | 35- 44 | 33 |
| 4-6 months | 60 | 23 | 37 |
| 12 months | - | 26-30 | - |
| Puberty | 60 | 20 | 40 |
| Adult | 50-60 | 20 | 40 |

Cited from Reed M 1996 Sage Publications [101]

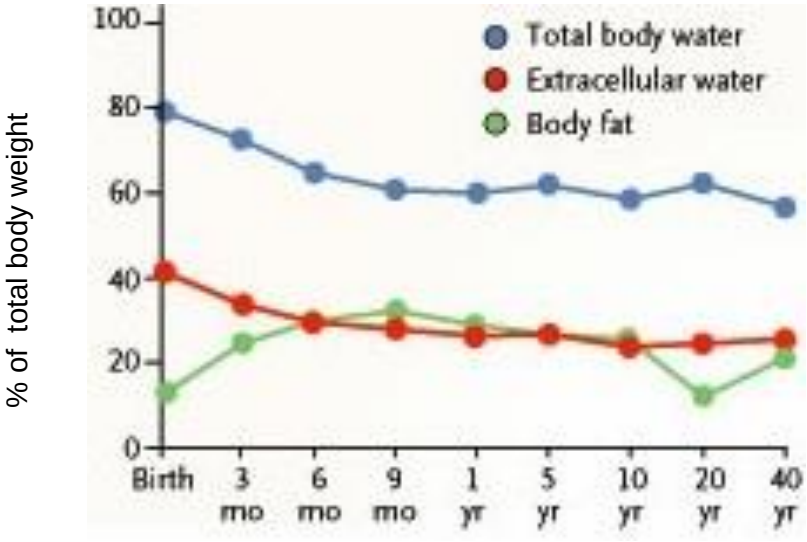



Figure 1-7 Change in distribution sites with age

This figure compares the change with age in total body water, extracellular water and body fat as a percentage of body weight [78].

1.1.5.2.1 Lipophilic and Hydrophilic Drugs

Antimicrobial agents may either be primarily hydrophilic or lipophilic according to their solubility. Ciprofloxacin is lipophilic resulting in a large volume of distribution with lower plasma concentrations whereas hydrophilic drugs remain in the extracellular space and are at greater risk of inter- and intra-individual variability [88]. Lipophilic drugs are primarily distributed in tissue therefore the influence of age on the apparent volume of distribution may not be as readily apparent compared with hydrophilic drugs that are influenced by changes in fluid [78, 102]. Membranes primarily composed of lipid can be classified in two groups: polar (phospholipids and glycolipids) and neutral (sterols and mono-, di- and triglycerides) [75]. The drug passes through a lipid barrier and enters the central compartment from which the drug again passes through another lipid barrier and enters the liver, kidney, tissue or plasma. It may then pass

via biliary excretion into the gut or undergo urinary excretion [75].

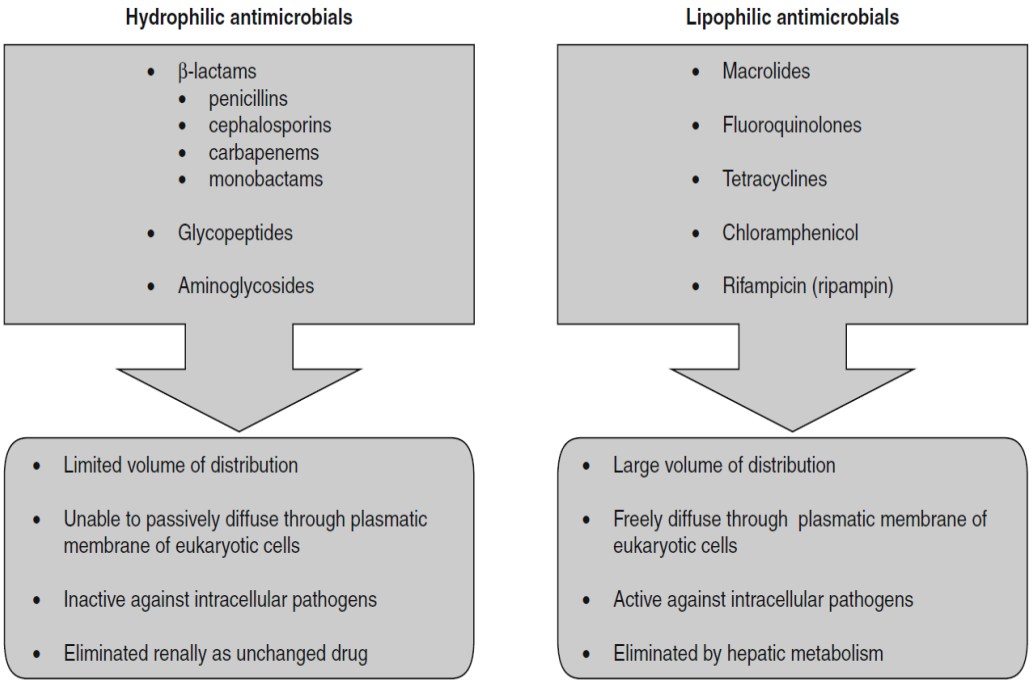



Figure 1-8 Classification of antimicrobials according to solubility
Reproduced from Pea et al Springer Publications [102]

As infants have a higher proportion of body fat than adults, they may have a larger weight adjusted volume of distribution for lipophilic drugs. Over the period of sepsis changes in volume of distribution and clearance may affect the antibacterial concentration at the target site. Pea *et al.* suggested that increased doses may be required for hydrophilic drugs in sepsis [88]. However, lipophilic drug concentrations are less likely to be influenced by variations in extracellular fluid during sepsis [103]. PK variations for gentamicin were studied in critically ill medical adult patients (n=40), suffering Gram-negative sepsis. The apparent volume of distribution (Vd) on the second day was 0.43± 0.12L/kg while on the seventh day of treatment it was 0.29+-0.17 L/Kg [104]. Roberts *et al.* reported increased volume of distribution consistent with changes in extracellular water with a decreased $C_{max}$ during sepsis for most antimicrobials including aminoglycosides, B Lactams, carbapenems and vancomycin.

In contrast, the volume of distribution for fluoroquinolones was unrelated to changes in extracellular fluid [86]. Lipman *et al.* found that the fluid shifts in adults with intra-abdominal sepsis and ascites did not alter the distribution of ciprofloxacin [105]. Ciprofloxacin is lipophilic, therefore has a greater affinity for intracellular distribution, and is less likely to be affected by changes in volume of distribution associated with fluid shifts [88, 89].

#### 1.1.5.2.2 Protein Binding

Drug distribution is affected by alterations in the protein binding. Albumin is the serum protein responsible for the majority of drug binding and may be altered in critical illness. Drug concentrations are affected by fluctuations in protein during the sepsis episode [93, 106]. Decreased binding may result from the co-administration of other drugs that compete for binding sites or hypoalbuminaemia [89]. Most drugs are partially bound to protein in the bloodstream; the unbound fraction exhibits the pharmacological effect that can then be metabolized and/or excreted. It is only the unbound fraction of a drug that is available for elimination from the vascular compartment and distribution into tissues or exerting a pharmacological effect [107-109]. When the bound fraction is released from the protein the free drug may then be eliminated from the plasma [75]. The bound fraction of the drug acts as a reservoir increasing the half-life but when it is bound the availability of active drug is lower [89, 93, 109]. In neonates albumin is lower at birth (35g/L) rising slowly through adult life to 45g/L [93, 106]. The reference range at Alder Hey Children's NHS FT and Liverpool Women's NHS FT for albumin in babies 0 - 2 months age is 30-45g/L and > 2 months 37-53 g/L but does not include a range for premature babies [93]. However, Ciprofloxacin is only moderately bound to protein 20-40% therefore may vary less in relation to changes in albumin [53].

Neonatal maturation may influence the distribution of the drug into tissues and anatomical compartments. Ciprofloxacin has properties that enhance the penetration of the blood brain barrier. The entry of drugs, including antibiotics, into the cerebrospinal fluid and extracellular space of the brain is governed by the molecular size of the compound, protein binding, active transporters and in particular lipophilicity (as the whole central nervous system is surrounded by lipid layers) [110]. The ability of drugs to penetrate the CNS is clinically important when treating meningitis. Transport across the blood brain barrier occurs by both passive diffusion and by active transportation [79]. Altered distribution of active substances or metabolites into the central nervous system (CNS) may have a potential impact on both clinical efficacy and adverse effects.

Cerebrospinal fluid (CSF) drug concentrations are often higher in infants and the elderly than in children and young adults [111]. Depending on the pathogen responsible and the severity of disease, CNS infections cause an increase in the permeability of the blood-CSF/blood-brain barrier and/or a decrease of the CSF flow. This frequently leads to an increase of drug concentrations in the central nervous compartments during inflammation [112]. The $AUC/_{CSF}$ v $AUC_{\text{steady state (s)}}$ ratio for ciprofloxacin has been reported as between 0.24 and 0.43 when the meninges are not inflamed increasing to 0.92 when inflamed [111, 113].

Ciprofloxacin is moderately lipophilic therefore of great value for the treatment of CNS infection by Gram-negative aerobic bacteria. The CSF of neonates contains high concentrations of proteins transferred from blood across the epithelial cells of the immature choroid plexus, and permeability to small lipid-insoluble molecules is greater in the developing than in the mature brain [114]. The penetration into the CSF in the absence of meningeal inflammation is much higher than that of β-lactam antibiotics based on the $AUC_{CSF}/AUC_S$ ratio. However, because of the relatively high CNS toxicity of fluoroquinolones it is not feasible to increase the systemic dose as

with β-lactam antibiotics [110]. Any medicinal product interacting with glutamic acid and other neurotransmitters may have an effect on neonatal brain development [79]. This is unknown for ciprofloxacin but there has been a report of fitting associated with higher $C_{max}$[115]. The neonatal blood brain barrier is not fully mature so medicinal products and endogenous substances such as bilirubin may gain access to the CNS with resultant toxicity [80]. Neonates also have increased concentrations of fatty acids and bilirubin that competitively bind to albumin [93].

#### 1.1.5.3 Metabolism

Drug metabolism or biotransformation refers to the process of modifying or altering the chemical composition of the drug. The majority of the metabolites are pharmacologically inactive. Most metabolites have increased water solubility compared with the parent, which facilitates excretion [116]. Hepatic clearance depends upon hepatic blood flow, plasma protein binding, cellular uptake, hepatic metabolism and biliary excretion [79, 117]. The determinants of hepatic clearance of drugs may be influenced by sepsis and septic shock [118].

Children may have an enhanced capacity to metabolise drugs due to proportionally larger livers and kidneys than their adult counterparts [74]. They have higher weight adjusted clearance. Until about six months age the liver is immature and may lead to high plasma concentrations of drugs metabolised by the liver [93]. The metabolic processes that promote drug excretion from the body are classified as Phase 1 or Phase 2 reactions Figure 1-9.

**Phase 1:** oxidation, reduction, and hydrolysis reactions are when cytochrome P450 'CYP' enzymes alter the molecule to form a more polar metabolite. Cytochrome P450 enzymes are essential for the metabolism of many medications and detoxification of chemicals: this class has more than 50 enzymes, six of which (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5) metabolize 90 percent of drugs [119]. They can be inhibited or induced by drugs, resulting in clinically significant drug-drug

interactions that can cause unanticipated adverse reactions or therapeutic failures. Knowledge of the most important drugs metabolized by cytochrome P450 enzymes, as well as the most potent inhibiting and inducing drugs, can help minimize the possibility of adverse drug reactions and interactions.

**Phase 2:** glucuronidation, sulfation, acetylation, and methylation which conjugate the metabolite with another, often more water soluble hydrophilic molecule, allows renal excretion of phase 1 metabolites that are not sufficiently polar and less lipid soluble than the original drug [93].

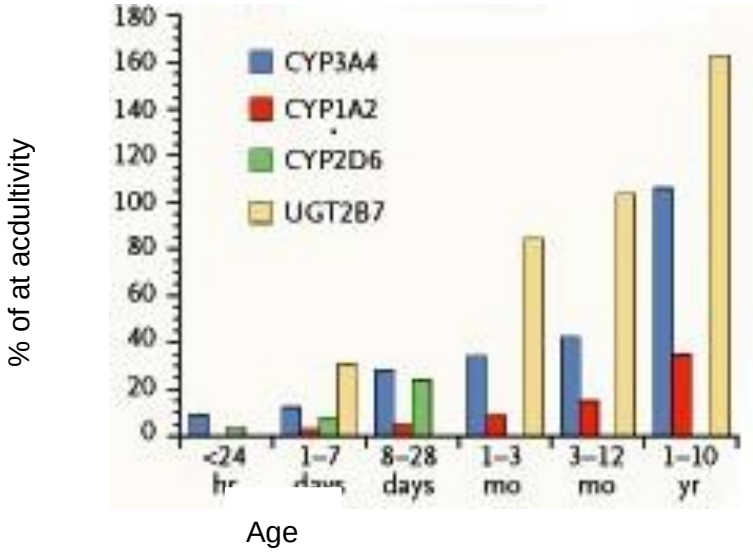



Figure 1-9 Change in metabolic capacity with age
In this figure the CYP enzymes are Phase 1 and the UGT287 is Phase II [78]

Ciprofloxacin inhibits CYP1A2, therefore concomitant administration of drugs metabolised by this enzyme may result in increased serum concentrations of the concomitant medicine resulting in adverse reactions [53, 102]. Drugs relevant to ciprofloxacin that are commonly prescribed to neonates include caffeine, theophylline, ondansetron and propranolol [53].Figure 1-9 illustrates that CYPIA2 is significantly reduced in children <28 days and remains low until one year of age. The EMA advise that if the drug investigated is likely to be eliminated mainly via hepatic metabolism,

markers of hepatic function should be included as covariates in the analysis and safety assessment [79]. (*see section 1.3.1* specific to ciprofloxacin)

#### 1.1.5.4 Elimination

Elimination of renally cleared drugs is dramatically reduced in the newborn compared with older infants and children because of reduced glomerular filtration and tubular secretion [83]. Variability in elimination may lead to either the risk of over-exposure increasing toxicity or under-exposure leading to therapeutic failure [75, 88]. The pharmacokinetics of antibiotics that are primarily eliminated renally will vary considerably in neonates due to their immature renal excretory function [93].

Elimination and distribution to target sites might be influenced by active drug transporters. Ciprofloxacin is transported by organic anion transporter 3 (OAT3) and by the breast cancer resistance protein (BCRP) transporter, both transporters being genetically polymorphic. Thus, individual differences in efficacy and in elimination might partly depend on genetic polymorphisms of active drug transporters [57, 120, 121].

Nephrogenesis is essentially complete at approximately 34 weeks of gestation. Glomerular Filtration Rate (GFR) increases rapidly after birth from 10 mL/min/1.73 $m^2$ to 20-30 mL/min/1.73 $m^2$ in the first two weeks of life then rises steadily until adult values are reached between 8 and 18 months of age [78, 79, 122] Figure 1-12. Renal function is limited by poor peritubular blood flow, reduced urine concentrating ability and lower urinary pH values [79]. Post-natal changes occur in renal blood flow which increases from 12 mL/min at birth to 140mL/min by two years of age [123]. Unbound drugs can enter the glomerular filtrate where they may be excreted into the proximal tubule and reabsorbed by a passive process that is largely governed by the physiochemical characteristics of the drug (e.g. lipid solubility, ionization) [93]. When medicinal products such as ciprofloxacin are excreted via active tubular secretion premature neonates are at a higher risk of toxicity therefore require special attention.

The primary covariates for clearance often include weight or surface area. Weight can under-predict clearance and the surface area model over-predicts clearance in children [124, 125]. Pea et al. suggest that creatinine clearance is the best surrogate of glomerular filtration rate to monitor renal function and adjust dosage [102]. However, there are limitations as creatinine may be elevated during the first week of life due to the persistence of maternal creatinine [79, 83, 95]. Therapeutic drug concentration monitoring may be required due to toxicity as a result of reduced clearance in neonates as shown with gentamicin [126]. In very low birth weight (VLBW) babies this elevated serum creatinine may continue for the first few weeks of life [75]. Lower post menstrual age (PMA = age calculated from 1st day of last menstrual period) at birth usually results in a higher serum creatinine [123]. GFR correlates better with post menstrual age than post-natal age (PNA = calculated from the first day of birth) [79]. This may also depend on how GFR is calculated, if corrected for body surface area then GFR increases more slowly to reach adult concentrations between one and two years of age [79].

Vieux et al compared Glomerular Filtration Rates for neonates born prematurely between 27 and 31 weeks gestational age (GA) for the first four weeks of life. They found that a neonate born at 27 weeks had a GFR of 18 mL/min/1.73m$^2$ at 31 weeks corrected age whereas the GFR for a neonate born at 31 weeks was considerably higher 25 mL/min/1.73m$^2$25 [127]. This suggests further delays in nephrogenesis for premature babies during the post natal period therefore dose regimen may need to be adjusted for prematurity rather than the corrected post natal age.

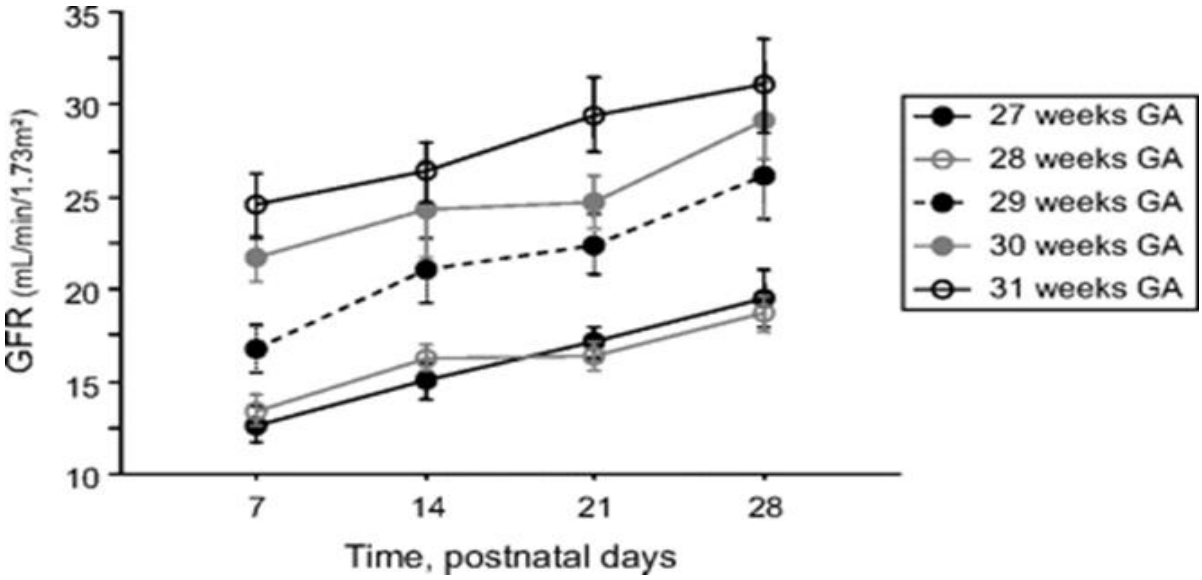



Figure 1-10 Glomerular Ffiltration Rate (GFR) for premature neonates at birth (Gestational age) compared to post natal age over the first four weeks of life [128]

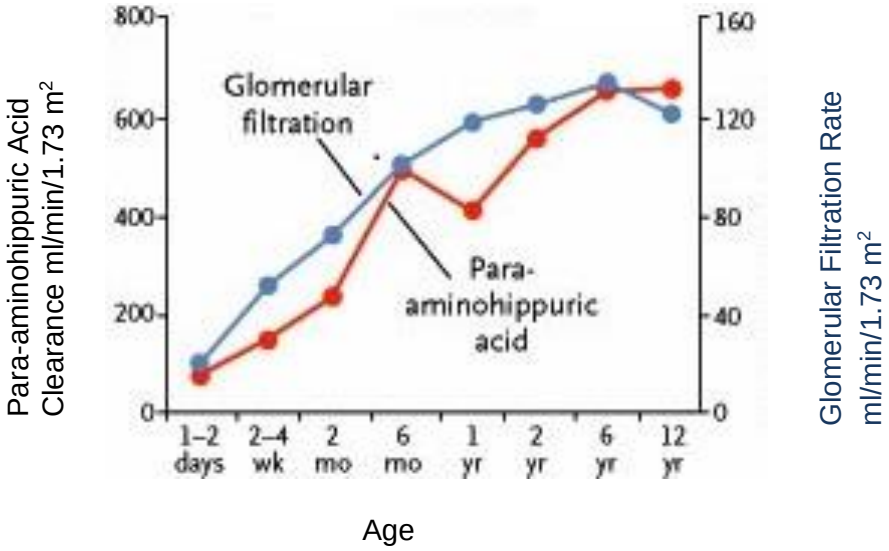



Figure 1-11 Renal Function and Glomerular Filtration Rate with age [78]

Para-aminohippuric acid is used for the measurement of renal plasma flow because it is secreted primarily by the renal tubules; only 20-30% is filtered by the glomerulus [101].

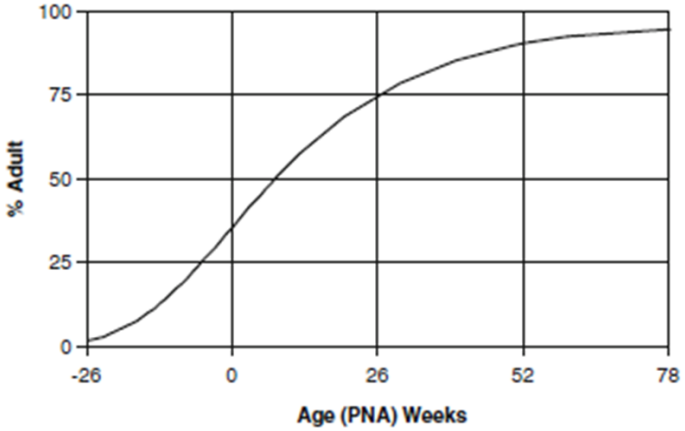



Figure 1-12 Maturation of GFR with post-natal age (PNA) compared to adults

Reproduced from Rhodin et al 2009 Springer Publications [122]

Factors associated with sepsis may either increase or decrease clearance. Clearance may increase due to hypermetabolic conditions or with administration of inotropes as the cardiac output increases enhancing renal blood flow [88]. Pea *et al.* found vancomycin concentrations decreased during co-treatment with inotropes and attributed this to the increased cardiac output and renal blood flow [129]. In severe sepsis clearance of ceftriaxone almost doubled compared with healthy adult volunteers but was only attributed to inotropes for 3/10 patients [91]. Lipman observed high ciprofloxacin clearance in one patient and suggested this may have been related to the hyper-dynamic condition during sepsis [105]. Hypoxia (at birth or later), congenital cardiac defects, septic shock and concomitant drugs (for example anti-inflammatories) may influence both renal excretion and or hepatic failure in neonates [79]. The cytochrome P450 enzyme system is an important clearance mechanism for many drugs (section 1.2.2.2), and has been shown to be markedly affected in septic conditions, resulting in decreased clearance [118].

In summary, PK-PD studies of neonates with critical illness and suspected sepsis are particularly challenging. Factors influencing the pharmacology of ciprofloxacin result in both inter and intra individual changes that are dynamic throughout their development and the disease process (Figure 1-13).

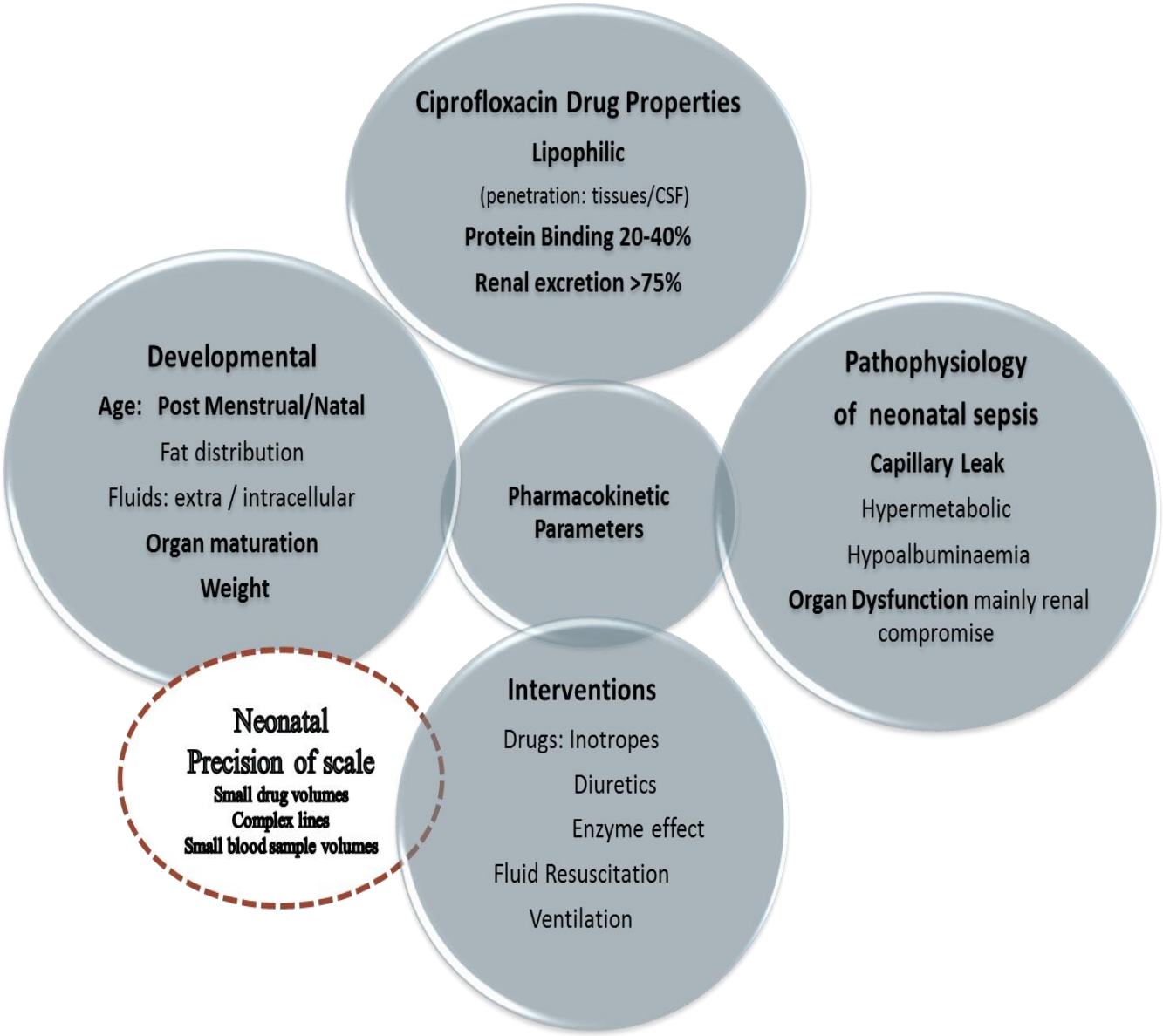



Figure 1-13 Developmental and pathophysiological factors influencing PK parameters of critically ill neonates

### 1.1.6 Population Pharmacokinetics (PopPK)

Population pharmacokinetics is the study of the variability in PK parameters between individuals. This approach quantifies variability in terms of patient characteristics such as age, renal function, sex, weight or disease state as well as residual and unexplained variability [130]. In traditional PK inter-individual variability is often deliberately minimised through restrictive inclusion/exclusion criteria but in population PK the extent of interindividual variability is of primary interest [131]. Sparse or rich data can be used [130]. The population PK approach differs to the traditional pharmacokinetic evaluation as it includes the following features [132]:

- PK parameters in patients representative of the population to be treated with the drug (using sparse or rich data)
- Quantifies population mean parameter values and the variability across a population of patients.
- Explanation of the variability by identifying factors including demographic, pathophysiological, environmental or concomitant drug-related origin
- Quantitative estimation of the unexplained (random) variability in the population
- inter-individual variability distribution (between subject variation)

The parameters required to predict concentration (C) at any time (t) may include the volume of distribution (V) clearance (CL), inter-compartmental clearance (Q) and an absorption rate constant (ka) (or absorption half-life) depending on the context [74]. PopPK was originally intended for monitoring drug concentration in clinical practice but are now used almost exclusively for phase II and III studies [133]. EMA Guidelines for PK in the paediatric population state that population PK analysis, using non-linear mixed effects models, is an appropriate methodology from a practical and ethical point of view [73].

### 1.1.6.1 PopPK Sampling Strategy in Neonates

Sparse sampling requires fewer samples instead of conventionally designed PK studies that requires between six to ten samples (per patient per dosing interval following the first or a steady-state dose). Fewer samples may still enable the fitting of unbiased models particularly if collected at maximally informative times. Even a single sample in a population design may provide reliable data. Forrest et al reported that as few as three to five samples can provide precise AUC and plasma clearance data [134]. Long et al collected 5 - 8 PK samples for netilmicin and ceftazidime and analysed the data for comparison with two, three or four time points. They concluded that three time points were sufficiently accurate for practical purposes in neonates [135].

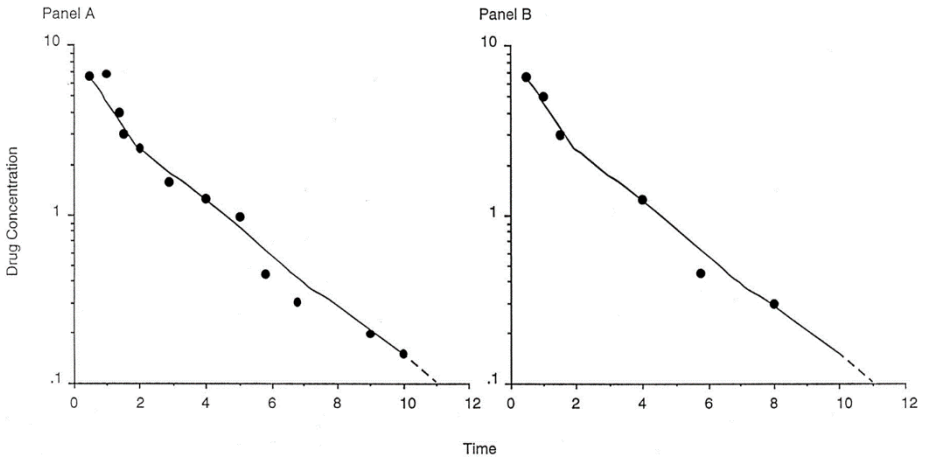



Figure 1-14 Typical drug concentration–time curve.

Construction of a drug concentration–time curve from a traditional, intensive sampling strategy (e.g. n = 12 samples, A) compared with an identical drug concentration–time curve constructed with sparse sampling (n = 6 samples) using optimal sampling strategy. Reproduced from Reed et al 1999 American Academy of Pediatrics [83]

The frequent blood sampling associated with traditional rich PK data is difficult in critical illness due to restrictions on the volume of blood taken from neonates for research purposes to minimise the risk of anaemia, pain, distress and disrupted sleep [136-139]. The EMA guideline recommends no more than 3% of total blood volume is collected during a four week period and no more than 1% at any single time [79].

Neonatal total blood volume is approximately 80-90 mL/kg. Very premature babies weighing <500g, may be restricted to as little as 1.5 mL sampling volume during a four week period [140].

Parameter estimates obtained at optimal sampling times have significantly less variability than those generated using the conventional sampling procedures therefore fewer samples are needed [141]. The optimal sampling time (OST) has traditionally been informed by *a priori* data from rich sampling data to indicate the information rich areas of the drug concentration time curve [142].This strategy is recommended by the EMA and American Food and Drug Administration (FDA) for neonatal PK data due to the severe limitations on samples [80, 131].

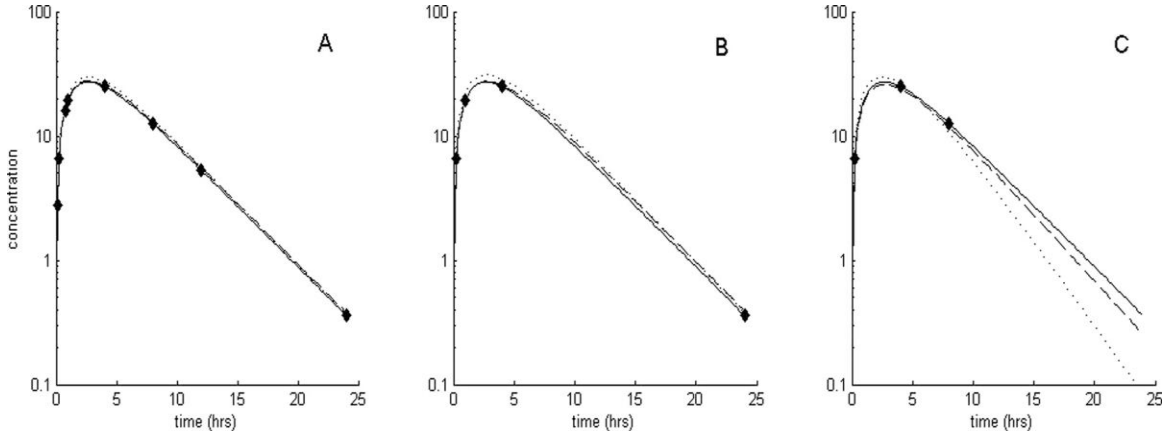



Figure 1-15 Concentration versus time curve for informative data

Hypothetical concentration versus time curve describing 'A' informative data with full sampling 'B' informative data with limited sampling, 'C' non informative data. The observed data are represented by the diamonds, the simulated model curve is represented by the solid line and the Bayesian fit curve with appropriate priors is represented by a dashed line and with inappropriate priors is the dotted line [143].

An OST strategy is dependent on the availability of a priori data from previous subjects including specific PopPK parameter estimates (e.g. AUC, V, CL, Q) [83, 141]. Preliminary *a priori* data are not usually available for neonates. To some extent the sampling strategy can be informed by the drug's behavior to identify which portions of the plasma concentration time curve provides the greatest information. This may

include the estimated time between administration, elimination and half-life but is less informative than *a priori* data Figure 1 14. Another option used in this trial is to collect sparse samples from two groups at alternating time points to produce a complete profile similar to rich PK data but with samples from the population.

In critical illness frequent blood samples are required for clinical care, often includes daily haematology and biochemistry in addition to four–six hourly blood gas analysis. These samples may be scavenged for further PK analysis if the precise time of sampling and drug administration are reliably recorded. One limitation is the need for all members of the clinical team to provide precise data on the drug administration and timing of samples. More than 200 clinical staff work in each intensive care unit at Liverpool Women's NHS FT and Alder Hey Children's NHS FT. The time the drug reaches the vein may be complicated by the small drug volumes, internal dead space of central lines or syphoning of drugs between connected lines. Often the time the drug is prescribed is simply signed rather than recording the actual time the drug was administered. Similarly, the time the blood sample is collection for clinical purposes is not routinely recorded with the precision required for PK analysis. To scavenge samples reliably requires the support of the wider clinical team.

The number of neonates recruited to a PK clinical trial aims to represent each month of development as the data will ultimately contribute towards licensing the drug for each sub-age group [79]. The appropriate number of patients will also depend on the number of subject specific characteristics that need to be evaluated [144]. Tam *et al.* found reasonably robust PK predictions required a population size of ≥ 50 [145]. Most neonatal studies have reported <20 patients, they are limited by their capacity to identify the predictors of altered PK parameters.

#### 1.1.6.2 PopPK Modelling

PK-PD modelling seeks to quantify variability in the data, and then simulate the predicted behaviour of the drug in the system [146]. PK model building is the application of statistical techniques to mathematically describe the patient outcome as a function of measures of drug exposure and other patient covariates [147]. The strengths and weaknesses of different methods are summarised in Table 1-5. A mathematical model is constructed to describe the data and the parameters that influence the model including absorption, distribution, metabolism and excretion. A PopPK data analysis can be performed using a number of packages such as NONMEM which was developed by Shreiner and Beal *et al.* [148, 149].

PopPK models determine the variability in drug response among the population representative of those for whom the drug will be used clinically. Estimating individual parameters for each subject is replaced by estimating a single mode then factoring in an inter-individual variability estimate for each parameter. This population information may then be used to improve the estimates of individuals with missing data, for example neonates who have only one PK sample. In addition the model allows data from several studies to be pooled to provide a single robust analysis [150].This is particularly valuable for neonatal studies as large sample sizes are rarely achieved.

NONMEM – Non-linear Mixed Effects Modelling is a software programme that determines the clinical variables that have a significant effect on the drug concentration for inclusion in the final model [149]. NONMEM assesses the effect of covariates then provides an estimate of the inter-individual variability by minimising the difference between the observed and predicted parameters [149]. Because the distribution of PK variables is random a nonlinear regression estimation method is required. A mixed effect model assesses the effect of random and fixed effects (age, weight, creatinine clearance). The influence of between subject variability (BSV) and within subject variability (WSV) on the observations i.e., plasma concentrations are

simultaneously quantified. The inter-subject variance model describes the between subject variability (BSV) on parameters that can be estimated from the standard deviation of the individual estimates. The criteria for selecting covariates for inclusion in the model is pre-specified and may use a step wise procedure forward inclusion 5% significance, and backward deletion 1% significance. To determine whether the inclusion of a covariate improves the significance of the model the objective function value (OFV) determines the probability of seeing the observed data. The smaller the objective functional value (OFV) number the less variability in the model, an OFV of >3.84 equates to a significance value of P=<0.05 and OFV of >6.635 to P=<0.01 based on chi square distribution [151].

The fixed effects and random effects represent the between subject variability and both values are assumed to be represented by parametric distribution. Exploratory analysis examines distributions and correlations among covariates and this may include a scatter graph or histogram to examine the normal distribution for errors. Skewed data may indicate, for example, the under prediction of concentrations. Estimates from preliminary analysis can be graphed against likely covariates (age, weight, renal function, disease states) in search for trends that can be incorporated in the covariate model.

**There are five steps to model building:**

- **Structural model**
- **Fixed Effects - Covariates Analysis**
- **Random effects**
- **Residual unexplained variability**
- **Internal model validation**
- **Simulation to predict the effect in the individual by Bayesian methods and in the population with Monte Carlo Simulation**

| Concept | Strength | Limitations |
|---|---|---|
| **Naïve Pooled Data**<br>Time concentration data are pooled as for a single subject (scatter graph) | Provides mean concentration for a group of patients<br><br>Simple methodology | Provides no information on:<br>Influential factors (age, weight etc.)<br>Individual profiles<br>Subject variability<br>Causes of variability/ standard deviation /IQR<br>Imbalanced data per individual could lead to biased estimates. |
| **Standard Two Stage Approach**<br>Stage 1 PK parameters for each subject 'individual plots'<br><br>Stage II Summary statistics | Determines individual patient parameters (e.g. V , CL)<br>Between subject variability can be estimated from the standard deviation (e.g. SD and IQR) and dispersion of central tendency from individual estimates (but only if there are sufficient rich samples)<br>The compartmental model provides more detailed parameter estimates | Requires rich data and precise time points<br>Between subject variability may be overestimated (no measure of error). Assumes individuals contribute equally.<br>Limited ability to accurately determine covariates this is dependent on rich samples (parameter estimates)<br>Summary data is influenced by outliers<br>Missing data could lead to biased parameter estimates. |
| **Population PK**<br>Models the variability in drug parameters among individuals and collectively<br>Fixed effects describe the predictable between subject variability (BSV)<br>Random effects describe the between occasion variability (BOV)<br>Explanatory covariates explain the predictable part of the between subject variability | Represents the population treated clinically<br>Provides a population average and individual parameters can be obtained using empirical Bayes estimates.<br>Dense or sparse data even a single sample can be used<br>Explores covariates<br>Imbalance in data per individual can be handled.<br>Data can be pooled across studies<br>The compartmental model provides more detailed parameter estimates<br>De-emphasises outliers - the mean and median are representative of actual values in the population<br>Sample timing can be flexible in line with standard care.<br>Quantifies the unexplained variability in the population | Parametric therefore assumes the normal distribution whereas the extremes in some populations may mean that parameters are not normally distributed<br>Covariates can exhibit co-linearity (e.g. age and weight)<br><br>Complexity of modelling requires specialised training and software |

Table 1-5 Pharmacokinetic Methods for Data Analysis

**Step 1- Structural Compartment Model**

Compartmental modelling defines drug disposition with greater specificity than is possible with a non-compartmental approach this includes clearance and the volume of distribution in each compartment. That is, it can describe the rate and extent of drug distribution and elimination from the blood, central and peripheral compartments by parameters that include central ($V_1$) and peripheral ($V_2$) and the inter-compartmental difference (Q). The first step of compartmental modelling is to determine the structural model, the number of compartments (usually one, two or three compartment) depending on the available data:

- One compartment – circulatory
- Two compartment – circulatory and tissue
- Three compartment – circulatory, tissue and deep tissue.

In an open one compartment model the drug is assumed to distribute instantly between the blood and other body fluids or tissues. A two compartment model is when the drug is not instantly distributed. The concentrations in each compartment can be predicted even though the actual compartment may not be accessible for sampling. The structural model is partly influenced by the availability of samples therefore sparse data may not be sufficient for a two compartment model. Figure 1-16 illustrates the data required for one or two compartment models

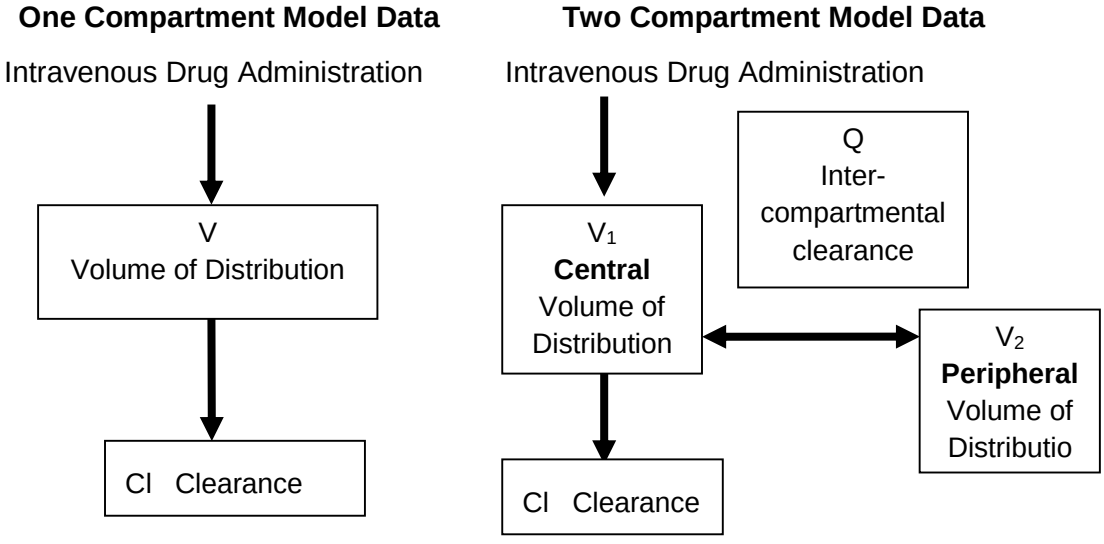



Figure 1-16 One and two compartment models

To determine which model is selected (one or two compartment) the concentration–time data is plotted on a semi-log plot to find the terminal slope; this is back extrapolated to the axis. If there is no concentration-time data above the line it is one compartment, otherwise it suggests a delay between the drug distributing into the tissues. The first graph represents a one compartment model with immediate distribution Figure 1 16 is compared to Figure 1 17 with a non-linear terminal slope.

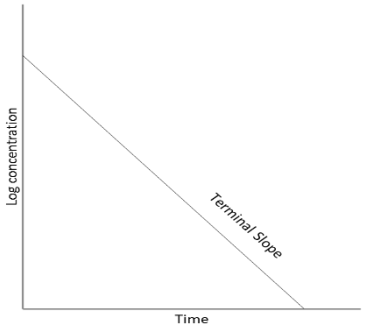



Figure 1-17 Intravascular Administration - One Compartment Model

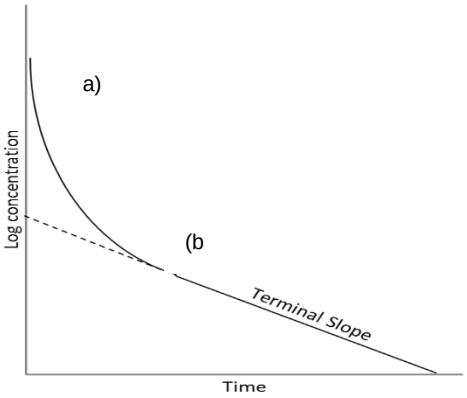



Figure 1-18 Intravascular Administration - Two Compartment Model

Once an injection has been completed (a), the drug concentration in the central compartment is highest. The concentration falls mainly because the drug is distributed from the central to the peripheral compartment (distribution phase) and partly because some elimination begins. At point (b) drug distribution in the central and peripheral compartments are approximately equal this is the steady state. At this point the main reason for the drug leaving the central compartment changes to the elimination process rather than distribution. At (b) the curve is back extrapolated and if there are data above this line then it is a two compartment model.

The distribution between compartments can occur at different rates but eventually a steady state will be reached terminating the distribution phase [75] Figure 1-19.

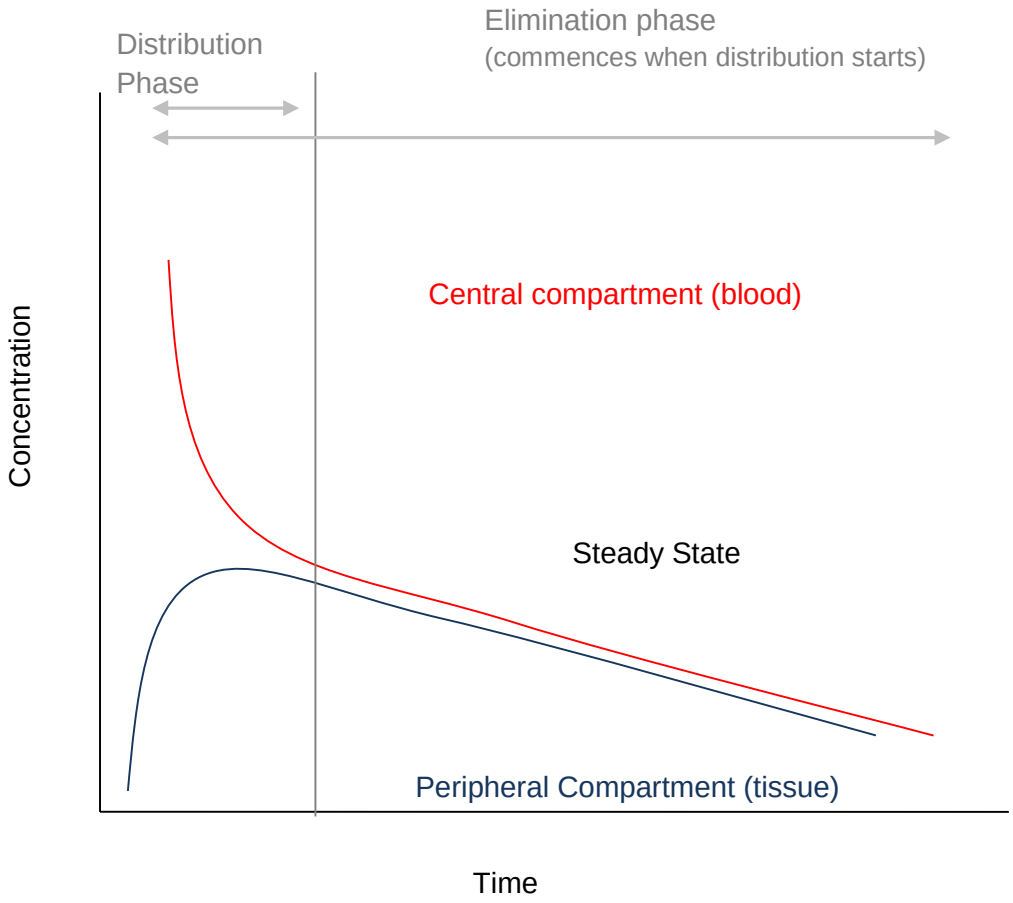



Figure 1-19 Concentration in central and peripheral compartments

As the concentration in the central compartment decreases the concentration in the peripheral compartment increases until steady state is reached. The distribution phase informs the volume of distribution between the $V_1$ central compartment and $V_2$ peripheral compartment. The elimination phase primarily provides data for clearance.

**Step 2 - Fixed Effects -** covariate analysis determines factors that influence the difference in parameters. Fixed effects on parameters are predictable known observable effects that occur across the population such as age and between subject variability (BSV). These define the average value for a parameter in a population and/or the average relationship between measurable patient factors (creatinine clearance) and PK parameter. The estimated parameters are labelled THETAS $^{\theta}$. Explanatory covariates (age size, renal function, sex) can be introduced to explain a portion of between subject variability. These include the known changes

associated with neonatal development including allometric changes such as weight or post menstrual age. Ciprofloxacin is mainly excreted renally therefore the immature renal function for neonates would be a fixed effect covariate. A limitation of NONMEM is that covariates can exhibit co-linearity. In paediatrics clearance may change as a function of age, weight and/or renal function all related with the maturation of the child, these are not mutually exclusive and may show a high degree of correlation. Bonate *et al.* recommended that when correlation is >0.5 not all covariates are important for inclusion in a model [133].

**Step 3 - Random Effects define both the error and residual unexplained variability (RUV).**

The error model estimates the difference between observed and predicted values. These are the population parameters **(**THETA $\theta$) that are unknown when the model is being built or unpredictable variability including:

- Between Subject Variability (BSV) and inter-individual variability that naturally occurs between people (OMEGA Ω).
- Residual unexplained variability (RUV) including within subject variability (WSV) or intra- individual variability (SIGMA Σ)
- Between occasion variability (BOV) is a measure of unexplained random differences in an individual between different occasions.

Residual unexplained variability (RUV) represents the uncertainty in the relationship between the plasma concentrations predicted by the model and the observed concentration. This is the difference between the parameters predicted by the model for the individual and the actual measurements observed. This may include model misspecification. This is intraindividual or within the individual subject variability. Random effects may be accounted for by assay errors (either a consistent error throughout the assay range or may increase proportionally), drug dose, or the time of measurement. Assay errors may be a consistent error throughout the assay range (additive) or as substrate increases (proportional). Residual error is reported as a

mean of 0 and the distribution, as positive errors cancel negative errors. When variation in the population is high this is recorded as Residual standard error RSE%.

**Step 4 - Internal model validation**

Model validation requires an assessment of covariates that are clinically relevant. A posterior predictive test simulates the data that contributed to the model. This includes PRED a prediction within the population and iPRED a posterior predictive check for the individual. These visual predictive checks provide reassurance that the model is capable of recreating the data from which the model was built. Basic internal evaluation of a model consists mainly of Goodness-of-fit (GOF) plots, which detect potential bias or problems in the structural model and/or the random effects models. GOF plots include population predictions (PRED) overlaid on observations (OBS) versus time. Simulated data from the final PK model are compared to the original data, 90% of predictions should encompass 90% of the observations [152].

Bootstrapping tests the reliability of the model by randomly simulating patients then recalculates the parameters including volume of distribution, clearance and inter-compartment clearance ($V_1$ and $V_2$). Data for patients are randomly sampled and modelled to see if the estimates are consistent. As many as a thousand iterations may be required to estimate 95% confidence intervals. If the bootstrap model matches, the model is robust. Reliability of the results of the analysis can also be determined by evaluating the precision of parameter estimates from standard errors (SE) or confidence intervals (CI).

**Step 5 Simulation**

Bayesian statistics is a method of updating uncertainty in the light of evidence when the initial beliefs about some unknown quantity are represented by a prior distribution. The population PK parameters produced by NONMEM may be explored using Bayesian prediction methods. The parameters for an individual with sparse data even

as little as one sample can be modelled to predict the individual dose regimens informed by prior distributions.

Monte Carlo Simulation is where a data set is simulated based on the known distribution of PK parameter estimates and subject variability. This mathematical model may be used to predict optimal dosing associated with the probability of a successful outcome in the treatment of an infection. The values from a limited number of patients can mathematically create an AUC of an antibiotic's distribution for many subjects. A pharmacodynamic model may then incorporate the AUC for a specific dose with the susceptibility of the pathogen to predict the probability of target attainment thereby informing clinical breakpoints. If the therapeutic target for the organisms is known the data can be used to determine the proportion of patients that will reach the target or predict a dose with a higher probability of attaining the target. Each pathogen or location in the tissue may have a different range of optimal AUC/MIC ratio.

### 1.1.7 Pharmacodynamics

Pharmacodynamics (PD) is assessing the outcome of a drug. Antimicrobial pharmacokinetics and pharmacodynamics (PK-PD) integrate both microbiology and pharmacology. This can either be the clinical outcome following systemic exposure and response to the drug, or the outcome of the organism either in the laboratory or the patient [76]. Rowland and Tozer [76] state this can be assessed as:

- a clinical response – 'subjective' e.g. patient's personal assessment or 'objective' e.g. mortality
- a surrogate endpoint
- a biomarker (a laboratory diagnostic or prognostic test).

The aim is to link a measure of drug exposure, relative to the susceptibility of the pathogen, to the microbiological or clinical effect achieved. This relationship allows the drug dose to be chosen in a rational manner so that the desired effect, for example maximum bactericidal effect, can be achieved in a large proportion of the intended

patient population [147]. Three main PK/PD predictors of efficacy have been established that depend on the antibiotic group [153]

Figure 1-20:

- Time above the MIC (T>MIC) expressed as a percentage of the dosing interval
- Ratio of the area under the curve over 24 hours to the MIC ($AUC_{24}$/MIC)
- Peak concentration after each dose to MIC ratio (Cmax / MIC)

PD studies of ciprofloxacin have established that the area under the curve $AUC_{24}$/MIC ratio is the optimal predictor of clinical outcome in adults and animal models [154, 155].

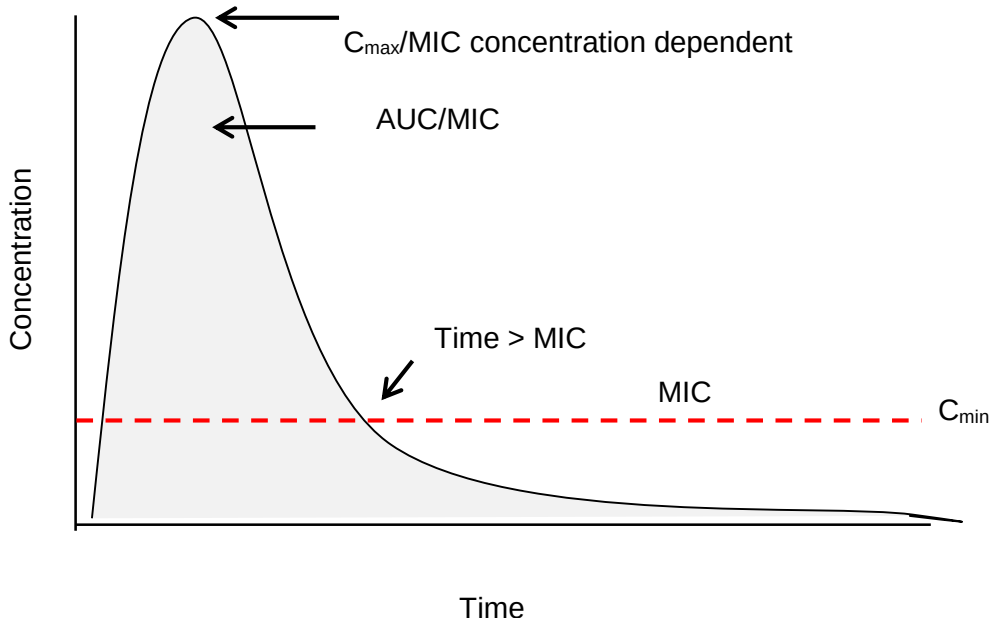



Figure 1-20 PK/PD parameters on a concentration versus time curve

The PK-PD parameters for antimicrobials AUC = area under the serum concentration time curve; Cmax = peak serum drug concentration; Cmin = minimum serum drug concentration; MIC= minimum inhibitory concentration; T>MIC = time for which the serum area under the inhibitory curve (AUIC) is when the actual inhibitory titres (blood concentration and MIC) have been measured simultaneously for a specific patient and used in the calculation [154].

Thomas et al. found antimicrobial resistance appeared to be strongly associated with suboptimal antimicrobial exposure and defined suboptimal as an $AUC_{24}$/MIC ratio of less than 100 [87]. Their study included ciprofloxacin and reported 82% bacteria with an $AUC_{24}$/MIC ratio <100 developed resistance compared to 9% above 100. Also, rapid resistance occurred below this ratio when 25% became resistant within 6 days. The authors claimed a $C_{max}$/MIC ratio of approximately 5:1 corresponded to an $AUC_{24}$/MIC ratio of 100 for ciprofloxacin.

#### 1.1.7.1 Clinical Breakpoints

Clinical breakpoint standards aim to predict which organisms will respond effectively to the antibiotic. They are set by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). In clinical laboratory routine practice the organism is cultured then tested against several antibiotics to determine whether it is susceptible or resistant using a rapid disc test. The Minimum Inhibitory Concentration (MIC) to inhibit growth of the organism may be quantified in the laboratory using the E Test. A microorganism is defined as susceptible 'S' when the MIC is low enough to be associated with a high likelihood of therapeutic success or as resistant 'R' when the MIC is raised and associated with a high likelihood of therapeutic failure. Intermediate 'I' implies some uncertainly, the antibacterial may be susceptible if concentrated at the site of infection (e.g. in urine) or susceptible if a high dosage of the drug is administered [153, 155, 156].

In the US the Clinical Laboratories Standards Institute expand these definitions by saying susceptible means the growth of the bacterial strain is inhibited by the usually achievable concentrations of antimicrobial agent when the recommended dosage regimens is used for that site of infection and antibacterial concentration in the range found for wild type strains [157]. The Etest is an *in vitro* measurement of the laboratory concentration of the drug required to inhibit bacterial growth. There is no definitive relationship between clinical breakpoints and the laboratory MIC (ECOFF) measured

by an Etest [153, 158]. The true value of the MIC is as a measuring tool that generates values to which PD endpoints and clinical outcomes can be reliably compared [153]. EUCAST publish clinical breakpoint tables that predict the probability of target attainment (PTA) for therapeutic success when treating organisms based on the known wild type MIC for the organism. At present clinical breakpoints are based on adult outcomes for adult dose regimens [159]. They are not informed by the usually achievable concentrations or outcomes of neonates or children. EUCAST's process of setting clinical breakpoints is illustrated in Figure 1-21. [156]

There are four main variables that inform the probability of target attainment (PTA) for therapeutic success:

i) Dose regimens
ii) PK parameters
iii) Minimum inhibitory concentration (MIC) of antibiotic
iv) PD parameters of the clinical outcome [155, 156, 160, 161].

Treatment success relates to the dose regimens, the site of infection, the species and clinical outcome response to infection specific to the population. Clinical breakpoints are determined by collecting clinical outcome data for patients then comparing the dose they were administered with the MIC of the organism.

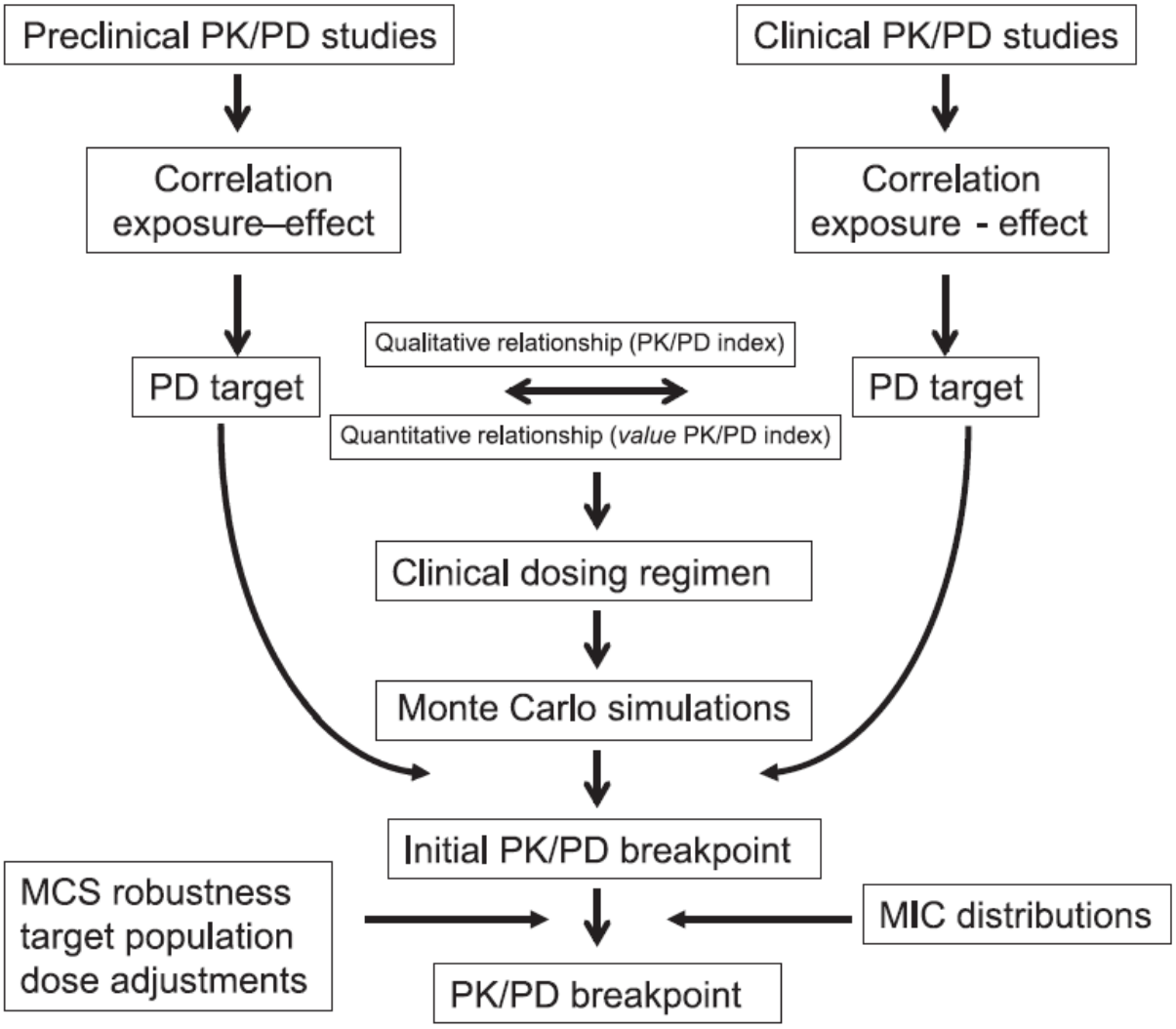



Figure 1-21 EUCAST's process of setting PK/PD breakpoints

Three definitions of cut off are defined by Turnidge *et al.* to clarify the difference between laboratory and clinical breakpoints [153]:

> **Epidemiological Cut off 'microbiological breakpoints** - defines the range for the wild type (WT) populations of bacteria as the upper limit of the MIC separating those with acquired, mutational or selected resistance mechanisms (based on large numbers of *in vitro* tests).
>
> **Clinical breakpoints 'Clinical cut off '' – the** MIC that separates strains that have a high likelihood of treatment success from those that are more likely to fail (based on a specific dose regimen).
>
> **PK/PD Cut off –**antibacterial concentrations derived from knowledge of a PD parameter and the dimension generated in an animal model then extrapolated to humans with a statistical model.

EUCAST define ciprofloxacin non-species specific clinical breakpoints as susceptible when ≤0.5mg/L and resistant ≥ 1mg/L these will render wild type susceptible including *Enterobacteriaceae*, *Pseudomonas* spp., *Acinetobacter* spp., *Staphylococcus* spp., *Haemophilus influenzae*, *Moraxella catarrhalis* and *Neisseria* spp. Different species are known to have different MIC and are provided in these tables however, in clinical practice non-species specific breakpoints or disc tests are used as the organism is not usually known at the start of treatment.

A clinical breakpoint of ≤0.5 mg/L allows *Enterobacteriaceae* to be categorised as susceptible to ciprofloxacin relating to an oral dose of 500 mg x bi-daily (BD) (or as low as 250 mg x 2 BD for uncomplicated urinary tract infections) to 750 mg x 2 BD and an intravenous dose of 400 mg x 2 BD to 400 mg x 3 TDS. The ECOFF for *Acinetobacter* is 1.0 mg/L is one of the less susceptible bacteria it has a higher clinical breakpoint of 1 mg/L to avoid dividing wild type MIC distributions therefore susceptibility for adults relates to the higher dosages of ciprofloxacin 400mg x 3 TDS [56]. For more severe infections EUCAST occasionally recommend either i) a lower clinical breakpoint 'MIC' ii) a higher dose (if tolerated). They are not related to neonatal or paediatric regimen or PK PD data.

Table 1-6 Epidemiological cut off (ECOFF) for ciprofloxacin and common Gram-negative species

| **Gram-negative species** | **ECOFF** 'wild type' MIC mg/L In vitro | **Clinical Breakpoint** MIC mg/L In vivo |
|---|---|---|
| *Acinetobacter* spp*. | 1.0 | <0.5 |
| *Citrobacter* spp. | 0.12 | <0.5 |
| *Enterobacter* spp. | 0.12 | <0.5 |
| *Escherichia Coli* | 0.03 | <0.5 |
| *Klebsiella* spp. | 0.06 | <0.5 |
| *Pseudomonas aeruginosa* | 0.5 | <0.5 |
| *Serratia* spp. | Insufficient evidence | |

*Source EUCAST [159]*
**Susceptible only for a higher dose regimen*

The clinical breakpoint relate to a specific dose regimen they indicate the likelihood of treatment success when the organism's growth is inhibited by a specific concentration using the laboratory MIC. The breakpoint can be higher, lower or the same as the MIC (ECOFF) Figure 1-23, Figure 1-24. If the regimen achieves low serum concentrations or low concentration at the site of infection it may only be effective for organisms with lower MIC. In this case the optimal MIC for cure would be lower than the ECOFF. In contrast a higher dose may achieve a concentration in the patient higher than the laboratory Etest when the breakpoint may be higher than the ECOFF. The important distinction is that there is no definitive relationship between clinical breakpoint MIC (concentration in the patient) and the laboratory ECOFF 'MIC' (concentration in the laboratory test) Figure 1-23and Figure 1-24. Organisms with MICs at the marginal point of susceptibility may subsequently develop resistance [162]. Falagas ***et al's*** reviewed the outcome with the MIC of the Gram-negative organism. Even when the MIC was within the susceptible range patients infected with strains with high MIC (RR 2.03 95% CI 1.05 to 3.92) particularly *P aeruginosa* and fluoroquinolones had higher all-cause mortality [163]. For non-fermentative Gram-negative bacilli mortality was higher with high MIC's was higher (RR 2.39; 95% CI 1.19 to 4.81) [163].

### 1.1.7.2 Mutant Protection Concentration

The optimal regimen may prevent the emergence of resistance if sufficient drug exposure adequately kills susceptible organisms however the efficacy of the concentration depends on whether it is tolerated by the patient. The mutant protective concentration (MPC) is defined as the drug concentration required to prevent all single step mutations in a population of at least $10^{10}$ bacterial cells in a population using wild type not resistant bacteria [48, 164]. The range between the MIC and the MPC is called the mutant selection window (MSW) Figure 1-22. Below the MIC there is no selective pressure as mutants are not selected therefore do not grow; above the MPC no mutants will be selected because it is thought that a double mutation for growth is necessary [48]. Above the MPC no mutants will be selected Increasing antibiotic exposure reduce the antibiotic colonies.

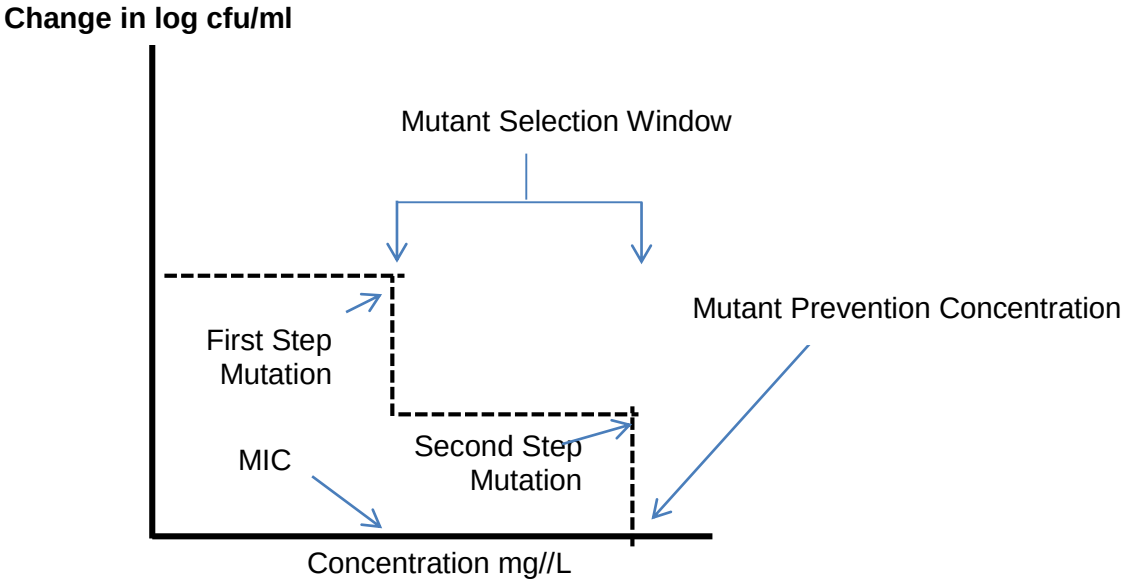



Figure 1-22 Mutant Selection Window and Mutant Prevention Concentration

Bacterial colonies reduce (cfu/ml) with increasing exposure to antibiotics. Above the MIC bacteria require a mutation to survive. An additional mutation is requierd to survive the second step. Selective antibiotic growth may occur when concentrations are inbetween these stages known as the mutant selection window. The mutant prevention concentration (MPC) requires at least a second step for bacteria to be resistant above this. (Adapted from Dong Y et al by Roberts et al [48, 165]

For colonies to survive the first step at the bacteria's MIC a second step mutation is required. Selective antibiotic pressures may occur when antibiotic concentrations are

in the mutant selection window. The optimal MPC requires a least a concentration above the second mutation for bacteria to survive [48]. For Ciprofloxacin Drusano *et al.* compared two drug doses producing AUC/MIC ratios 52 and 157 respectively; the suboptimal dose amplified the resistant subpopulation by 1.5 log10 (cfu/g) [147]. Roberts et al recommend a paradigm change to antibiotic selection and dosing strategies designed to consider limiting antimicrobial resistance by administering the highest tolerated dose of antibiotic. Olofsson et al found that neither T>MPC nor Cmax correlated to preventing resistance to *E coli* for ciprofloxacin, the $AUC_{24}$/mutant prevention concentration (MPC) was the optimal PD parameter to reduce resistance estimated as >22 [166]. For neonates the optimal regimen is unknown as further safety data is required [48].

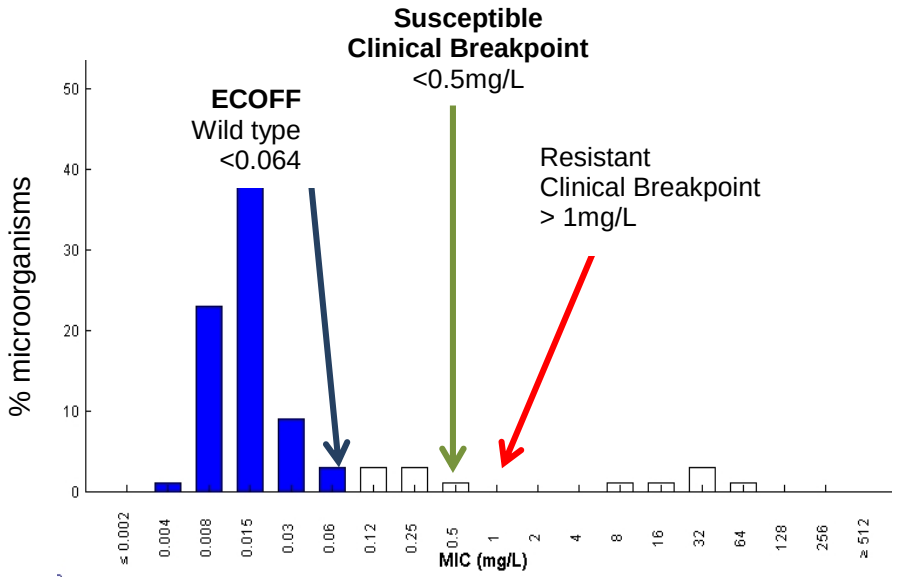



Figure 1-23 Ciprofloxacin and *E.coli* - ECOFF and Clinical Breakpoint

The ECOFF for *E. coli* is lower than the clinical susceptibility breakpoint based on the MIC distribution for 16702 observations reported in 2015 by EUCAST.

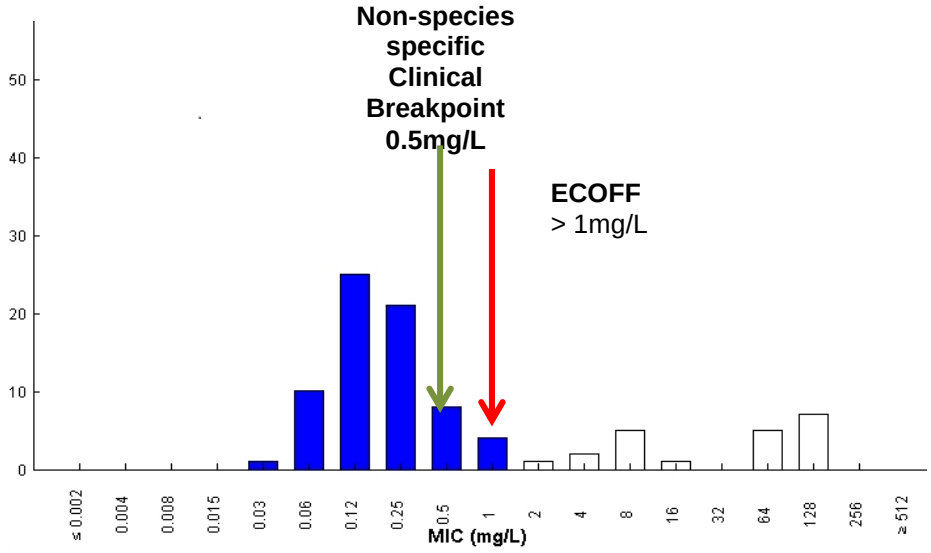



Figure 1-24 Ciprofloxacin and *Acinetobacter* spp. ECOFF and Clinical Breakpoint

The ECOFF is higher than the non-species specific clinical breakpoint for Acinetobacter MIC >1mg/L therefore it is only susceptible on a higher dose regimens of 400 mg x 3 daily in adults.
Both adapted with permission EUCAST [158].
http://mic.eucast.org/Eucast2/SearchController/search.jsp?action=performSearch&BeginIndex=0&Micdif=mic&NumberIndex=50&Antib=-1&Specium=227

## 1.2 Neonatal Antimicrobial Therapy

Prescribing practices may influence both the efficacy of the treatment and resistance development. Empiric antibiotic treatment is often initiated rapidly for suspected late onset sepsis due to the high risk of mortality and long term neurodevelopmental sequelae [11]. The frequent prescribing of broad spectrum antibiotics in past decades is now associated with the increase in multidrug-resistant Gram-negative bacilli (MDR GNB) [167]. Fernando *et al.* found that most NNUs (89%) used an empirical first-line antibiotic regimen for late onset sepsis (180). A quarter (28%) of units prescribed a broad spectrum antibiotic (e.g. third generation cephalosporin). Frequent use of third generation cephalosporins has been shown to increase resistance on neonatal units and outbreaks of extended spectrum β-lactamase producing bacteria [168, 169]. It is estimated that these resistant Gram-negative bacteria account for approximately 20% of bacteraemia cases and are associated with a 2.8-fold increase in neonatal mortality rate compared with non-MDR strains [167]. The use of third-generation cephalosporins is also associated with invasive fungal infections from which mortality is high [8]. The neonatal unit at Liverpool Women's Liverpool Women's NHS FT changed from the empiric use of cephalosporins over 25 years ago due to concerns for resistance to prescribing ciprofloxacin.

There is a lack of evidence to guide treatment of late onset sepsis reported by a Cochrane review [38]. In contrast, guidance for early onset sepsis is well established including National Institute for Health and Care Excellence (NICE) guidance (https://www.nice.org. uk/guidance/cg149 accessed January 2015). NICE are engaging with clinicians to develop evidence-based guidance for late onset sepsis http://www.nice.org.uk/guidance/qs75 /chapter/quality-statement-6-placeholder-antibiotic-treatment-for-lateonset-neonatal-infection (accessed January 2015). A review of antibiotic policies in British and Irish neonatal units found a consistent approach to prescribing for early onset sepsis but widespread random use of other

antimicrobial regimens for late onset sepsis Table 1-7 (unpublished with permission). Regimens frequently prescribed included co-administration of benzylpenicillin and gentamicin (35%), flucloxacillin and gentamicin (21%), ampicillin/amoxicillin and gentamicin (3%) [12]. Muller-Pebody *et al.* [71] reviewed 3,482 reports of bacteraemia and found that more than 95% of organisms (late-onset) were susceptible to gentamicin with either flucloxacillin or amoxicillin and amoxicillin with cefotaxime, but only 79% were susceptible to cefotaxime monotherapy. Monotherapy was used in only 8% of units [12]. Empirical second-line antibiotics for treating late onset sepsis, varied widely across the 46% units (93) that had second line policies, the most common regimens (cefotaxime and vancomycin) account for only 8% and other recommendations including meropenem, teicoplanin, piperacillin/tazobactam and aztreonam. Over one-third of the NNU policies (37%; 74 units) specified alternative antibiotics first-line for septic episodes where central venous catheters were in situ, most commonly vancomycin (21%) or teicoplanin (12%) [12]. Table 1-8 summarises antibiotics commonly prescribed for late onset sepsis [11, 12, 18, 38] .

Despite the challenges and lack of research in this population the EMA guidance for antimicrobial clinical trials is limited to the general population for drug development and has a brief paragraph in relation to paediatrics and neonates (discussed further in section 1.5.1) [79, 80].

Table 1-7 UK Neonatal Units Antibiotic Survey: Late Onset Sepsis ( n=202)

| | N= | % |
|---|---|---|
| **Ist Line Antibiotics (n=180/202)** | | |
| Benzylpenicilln and Gentamicin | 70 | 35 |
| Flucloxacillin and Gentamicin | 43 | 21 |
| Ampicillin/Amoxicillin and Gentamicin | 6 | 3 |
| Co-amoxiclav and Gentamicin | 2 | 1 |
| Benzylpenicillin monotherapy | 2 | 1 |
| Amoxicillin, Flucloxacillin and Gentamicin | 1 | <1 |
| Cefotaxime monotherapy | 2 | 6 |
| Cefotaxime and Amoxicillin/Ampicillin | 11 | 5 |
| Cefotaxime and Benzylpenicillin | 8 | 4 |
| Cefotaxime and Vancomycin | 6 | 3 |
| Cefotaxime and Flucloxacillin | 5 | 2 |
| Teicoplanin and Cefotaxime | 4 | 2 |
| Vancomycin and Gentamicin | 3 | 1 |
| Ceftazidime and Vancomycin | 3 | 1 |
| Piptazobactam and Vancomycin | 1 | <1 |
| Cefotaxime and Gentamicin | 1 | <1 |
| Amikacin and Flucloxacillin | 1 | <1 |
| Cefuroxime monotherapy | 1 | <1 |
| | | |
| **2nd Line Antibiotics NNU (n=93/202)** | | |
| Cefotaxime and Vancomycin | 16 | 17 |
| Flucloxacillin and Gentamicin | 12 | 13 |
| Cefotaxime and Gentamicin | 8 | 8.7 |
| Teicoplanin and Cefotaxime | 6 | 6.5 |
| Vancomycin monotherapy | 6 | 6.5 |
| Teicoplanin monotherapy | 5 | 5.3 |
| Piptazobactam and Vancomycin | 5 | 5.3 |
| Cefotaxime and Flucloxacillin | 5 | 5.3 |
| Teicoplanin and Gentamicin | 5 | 5.3 |
| Benzylpenicillin and Gentamicin | 4 | 4.3 |
| Ceftazidime and Vancomycin | 4 | 4.3 |
| Vancomycin in Gentamicin | 3 | 3.2 |
| Meropenem monotherapy | 3 | 3.2 |
| Cefotaxime monotherapy | 2 | 2.1 |
| Ceftazidime and Teicoplanin | 2 | 21 |
| Amikacin and Flucloxacillin | 1 | 1 |
| Ceftazidime and Gentamicin | 1 | 1 |
| Vancomycin and Azetreonam | 1 | 1 |
| Teicoplanin and Vancomycin | 1 | 1 |
| Teicoplanin and Meropenem | 1 | 1 |
| Co-amoxiclav and Gentamicin | 1 | 1 |
| Piptazobactam monotherapy | 1 | 1 |

*Personal communication and permission from Dr P Heath [12]*

Table 1-8 Properties of antibiotics commonly prescribed for late onset sepsis

| Antibiotic Class | Antibiotic | Spectrum | Susceptible Organisms | | Pharmacodynamics (in vivo) | | Main Excretion | Protein binding % |
|---|---|---|---|---|---|---|---|---|
| | | | Gram-negative (GN) | Gram-positive | Inhibition dependent | Parameter for clinical efficacy | | |
| Aminoglycosides* | Gentamicin | Broad | + (mainly GN excludes anaerobic | + Excludes streptococci | Concentration | Cmax : MIC | Renal | 70-85 |
| Fluoroquinolones | Ciprofloxacin | Broad ** if 3rd generation | + | + | Time | AUC $_{0-24}$ /MIC | Renal | 20-30 |
| Glycopeptides | Vancomycin | Narrow | No | + inc. *MRSA* | Time | AUC $_{0-24}$ /MIC | Renal | 55 |
| | Teicoplanin | | No | + | | | Renal | 90 |
| **B-Lactams** | | | | | | | | |
| Carbapenems | Meropenem | Very Broad | + | + | Time | %T>MIC | ] Renal | 2 |
| Cephalosporins 3rd generation | Cefotaxime | Broad | + e*xcludes pseudomonas* | + | Time | %T>MIC | Renal | 40 |
| Penicillins | Ampicillin | Broad | + | + *includes streptococcal* | Time | %T>MIC | Renal | 15-25 |
| | Benzylpenicillin | Narrow | No useful activity against *Pseudomonas* spp., *Acinetobacter* spp. or Enterobacteriaceae | + | Time | %T>MIC | Renal | 60 |
| | Amoxicillin **** Amoxicillin/Clavulanate | Broad | + | + | Time | %T>MIC | Renal | 17-20 |
| | Flucloxacillin | Narrow | | + excludes *Enterococcus faecalis* | | %T>MIC | Renal | 95 |
| | Piptazobactam | Broad | + | + | Time | %T>MIC | Renal | 30 |

** TDM as toxic to kidneys and 8th cranial nerve (penicillin co-administered to increase bacterial permeability)*
*** 3rd generation fluoroquinolones are broad spectrum but other fluoroquinolones are narrow*
**** Inactivated by penicillinases produced by Staph aureus and Escherichia coli*
***** Co-amoxiclav is a derivative of ampicillin*

### 1.2.1 Ciprofloxacin antimicrobial (fluoroquinolone)

Ciprofloxacin is a synthetic antimicrobial agent with broad spectrum activity against primarily Gram-negative but some Gram-positive organisms [53, 56]. Its mechanism of action is by the inhibition of two enzymes, type II topoisomerase (DNA-*gyr*ase) and topoisomerase IV essential for bacterial DNA replication, transcription, repair and recombination [53, 170]. It is a second generation quinolone; the first generation is nalidixic acid. It was synthesised by adding a fluorine at C-6 and a cyclic diamine piperazine at C-7 to add antimicrobial activity against Gram-positive and improved activity against Gram-negative but not against anaerobic bacteria [159, 171].

Ciprofloxacin is a lipophilic antimicrobial active against intracellular pathogens, this intracellular penetration results in extensive distribution through anatomical barriers including the blood brain barrier [172]. It has significant activity against *Pseudomonas aeruginosa* due to excellent bioavailability with good tissue penetration. Due to its lipophilic nature a high proportion is distributed intracellularly throughout body tissues and is present in plasma largely in a non-ionised form [53]. It reaches high concentrations in the lung, sinuses, inflamed lesions and urogenital tract where concentrations even exceed those of plasma.

Intravenous guidelines recommend infusing ciprofloxacin over 60 minutes [173]. Following intravenous infusion to adults the maximum serum concentrations were achieved at the end of the infusion and the PK parameters were linear [53]. Protein binding of ciprofloxacin is low (20-40%) [53]. Ciprofloxacin is largely excreted unchanged, primarily in the urine and faeces approximately 12.1% is excreted in the form of metabolites [53] (Table 1-9). After a single intravenous doses of ciprofloxacin 75% is eliminated by the kidney and 14 % in faeces compared to 55% renally and 39% in faeces for an oral dose [53].

Since ciprofloxacin is not extensively metabolized by enzymes a genetic polymorphism is not likely to significantly influence the overall efficacy or toxicity.

Elimination and target distribution might be influenced by active drug transporters as ciprofloxacin is transported by organic anion transporter 3 (OAT3) [121].

Table 1-9 Metabolites of Ciprofloxacin:

| | % of the IV dose excreted in: | | |
|---|---|---|---|
| | **Urine:** | **Faeces:** | **Biliary Route** |
| Ciprofloxacin (within 5 days) | 75% | 15% | 1% |
| Metabolites – low concentrations: | 9.5 | 2.6 | |
| $M_1$ - desethyleneciprofloxacin<br>$M_2$ - sulphociprofloxacin<br>$M_3$ - oxociprofloxacin<br>$M_4$ - formylciprofloxacin | | | |

Metabolites and the percentage of the intravenous (IV) dose known to be found in urine and faeces [53].

Only 10-20% of a single oral or intravenous dose is eliminated as metabolites (which exhibit lower activity than the parent drug). Four different antimicrobially active metabolites have been reported, Desethyleneciprofloxacin (M1), sulphociprofloxacin (M2), oxaciprofloxacin (M3) andformylciprofloxacin (M4). M2 and M3 account for one third each of metabolised substance and M1 is found in small amounts (1.3-2.6% of the dose). M4 has been found in very small quantities (<0.1% of the dose) [53]. .

Ciprofloxacin undergoes both glomerular filtration and tubular secretion; renal elimination takes place mainly during the first 12 hours after dosing. In adults renal clearance is between 180 - 300 mL/kg/h and the total body clearance is between 480 - 600 mL/kg/h [53]. The half-life of ciprofloxacin is 3-4 hours [53]. Severely impaired renal function in adults increases the half-life up to 12 hours. When serum creatinine is >124 µmol/L the dose is reduced to half and administered 12 hourly. When above 168 µmol/L it is administered 24 hourly [53]. Based on population PK analysis of paediatric patients with various infections, the predicted mean half-life in children is approx. 4-5 hours [53, 174]. Ciprofloxacin dosing in children with impaired renal and

or hepatic function has not been studied [53]. Non-renal clearance of ciprofloxacin is mainly due to active trans-intestinal secretion and metabolism. Ciprofloxacin is present in the bile in high concentrations <1% of the dose is excreted via the biliary route [53, 174]. When drugs are primarily eliminated by the kidney clinicians must individualise treatment regimens in an age appropriate fashion that reflects both maturation and treatment-associated changes in kidney function [78]. A lower dose of ciprofloxacin 5mg/kg /day is prescribed in this neonatal unit when renal function is impaired [134, 162] (Neonatal Late onset Sepsis Protocol – Liverpool Women's NHS FT).

#### 1.2.1.1 Ciprofloxacin Pharmacodynamics

Pharmacodynamic (PD) studies of ciprofloxacin are limited to adult data as there are no paediatric studies. The efficacy depends on the ratio of the total antibiotic exposure AUC/MIC (area under the concentration time curve at steady state over 24 hours/minimum inhibitory concentration representing the susceptibility of the organism. Ciprofloxacin is effective for Gram-negative and Gram-positive bacteria. Pre-clinical studies suggests a higher $AUC_{24}h$/MIC >125 is required to treat Gram-negative bacteria effectively than Gram-positive bacteria $AUC_{24}$/MIC >40 [162].

To optimise clinical and microbiological outcomes in adults targets of ≥125 are widely accepted mainly based on a study by Forrest *et al.* in 1993 [162, 175, 176]. Clinical cure was 80% when above an AUIC ratio of 125 compared to 42% when below (P<0.005) and microbiological cure 82% compared to 26% (P< 0.001) respectively [162]. The median time to bacterial eradication from respiratory secretions reduced to 1.9 days with an $AUC_{24}$/MIC ≥250 compared with 6.6 days when 125 - 250 and 32 days if <125 [162]. More recently, Zelenitsky *et al* found higher cure rates (91.4%) were attained with an $AUC_{24}$/MIC ratio >250 when compared to 28.6% when <250 [176].

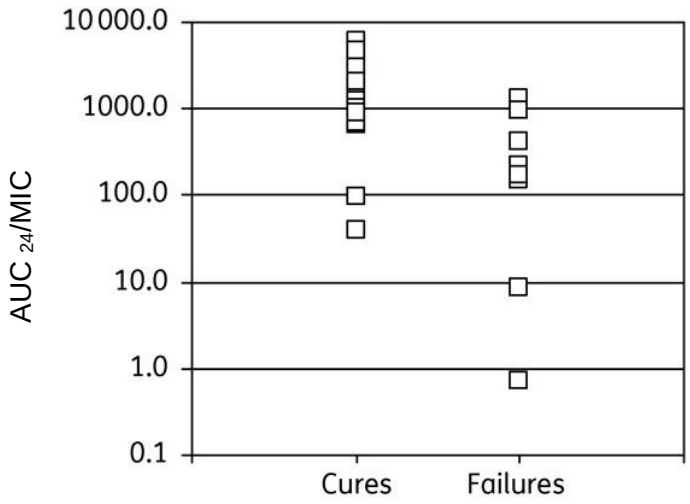



Figure 1-25 Ciprofloxacin a AUC $_{24}$ / MIC (mg/h/L) for adults

Figure reproduced from Zelenitsky *et al.* with permission from Oxford Journals [176]

#### 1.2.1.2 Ciprofloxacin Pharmacokinetics (PK)

Pharmacokinetic studies in children and neonates are summarised in Table 1-13 Lipman *et al.* [115] evaluated the PK of ciprofloxacin 20mg/kg per day administered 12 hourly for children comparing two age groups >3 months to 1 year (Group A) and >1year to 5 years of age (Group B) with severe sepsis (n=20). PK parameters were evaluated using non-compartmental methods. Areas under the curve (AUC, 12 h) were Group A: 15.6±1.3, 19.2±1.63 and 14.1±1.4 mg/h per L, and Group B 15.9±1.3, 18.0±1.7 and 13.2±1.26 mg/L. In infants the AUC ranged between 11.8 and 32.0 mg/h/L (mean 17.4mg*h/L) and children 11.0–23.8 mg/h/L (mean 16.5mg/h/L). The authors concluded that AUC were lower than achieved in their adult PK study. The paediatric PK data produced a lower AUC insufficient to achieve the minimum AUC ratio of >125 – 250 for an optimal outcome. The $AUC_{24}$ for Groups A and B would only be sufficient to treat organisms with an MIC of <0.3 mg/L and achieve a relatively low $AUC_{24}$/MIC ratio of 100 [115]. This is considerably lower than the EUCAST susceptibility clinical breakpoint of 0.5mg/L. This suggests 10mg/kg/12 hourly was a suboptimal dose and the authors recommended 10mg 8 hourly, particularly for more resistant ICU organisms in patients with normal renal function [115].

Szalek *et al.* found vast interindividual variability when ciprofloxacin was administered to critically ill patients. The standard adult dose of 400mg provided a lower $AUC_{24}$/MIC ratio than 125 for 70%, CV>30%; the Vd (l) increased, elimination rate constant was higher $0.21h^{-1}$, clearance higher 39.7 l/h [177]. Drusano *et al.* demonstrated that increasing the MIC and dose of ciprofloxacin were proportionate, as both AUC/MIC ratios had identical survival curves in rats. Isolates with an MIC of 1mg/L treated with 20mg/kg/day resulted in the same $AUC_{24}$/MIC ratio compared to when the MIC was 4mg/L and treated with 80mg/kg/day [178]. Different dose regimens produce variations in the probability of target attainment as demonstrated in Figure 1-24. Zelenitsky *et al.* found the significant factors for treatment failure were low ciprofloxacin $AUC_{24}$/MIC ($p<0.0001$), high MIC ($p=0.0001$), male sex ($p=0.002$) and low $AUC_{24}$ ($p=0.01$) [176].

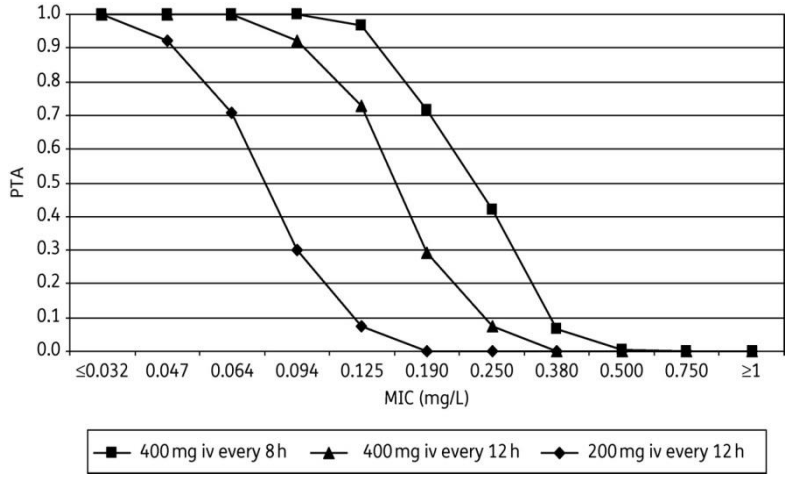



Figure 1-26 Ciprofloxacin MIC and probability of target attainment (PTA)

Probability of attaining an AUC24/MIC ≥250 in Monte Carlo simulations of 5000 study subjects with Enterobacteriaceae BSI stratified according to MIC. Zelenitsky *et al.* [176]. Reproduced with permission from © Oxford University Press

To optimise clinical cure and prevent spreading resistance a $C_{max}$ of 8 – 12 times the MIC ($C_{max}$/MIC) has been recommended for ciprofloxacin [179-181]. For critically ill septic paediatric patients the $C_{max}$ for children aged 3 months to 1 year was 6.1 ± 1.2 mg/l, mean clearance 3.7l/h (0.58 l/h/kg). A higher $C_{max}$ was achieved in children 1 –

5 years (7.4 +- 1.3), clearance 7.3 l/h (0.56 l/h/kg). Also, the $C_{min}$ was not maintained above the MIC for many organisms for much of the dosing period [115]. Forrest *et al.* compared Individual PK parameters values to the MIC to determine the peak ciprofloxacin concentration to MIC ratio, a ratio below four was associated with poorer clinical and microbiological outcomes. Their data suggest a peak-MIC ratio of eight and trough-MIC ratio above one were required to optimise outcome [162]. A $C_{max}$/MIC ratio of approximately 5:1 has been reported to correspond to an $AUC_{0-24}$/MIC ratio of 100 for ciprofloxacin [87].

CSF penetration of ciprofloxacin has been shown to vary but was approximately 22% of the serum concentration in an infant [182]. A case study of a neonate found CSF concentrations of 0.88–1.44 mg/L following a dose of 10mg/kg/day approximately 60% of the plasma peak [183]. Differences may be due to the presence or absence of inflammation.

### 1.2.2 Neonatal dose regimens

As ciprofloxacin is prescribed off label this has resulted in a wide range of dose regimen. A systematic review of ciprofloxacin in neonates reported the daily dose varied between 5 and 60 mg/kg/day and duration of treatment ranged from 5 to 75 days [184]. Ciprofloxacin regimens in Europe are summarised in Table 1-10. Existing PK data for this population is limited and summarised in Table 1-13. This variation in practice reinforces the need for PK data.

Table 1-10 TINN survey of dose regimens in European neonatal units

| Total dose mg/kg/day | Units | % (n=51) |
|---|---|---|
| ≤ 10 | 12 | (22) |
| 11-20 | 20 | (40) |
| 21-30 | 9 | (18) |
| >30 | 1 | (2) |
| Missing | 9 | (18) |

Paediatric PK data for ciprofloxacin recently led to a recommendation to increase the dose from 10mg/kg/day to 20 or 30 mg/kg/day for more resistant organisms [115]. In line with this, the neonatal 'off label' dose regimens was increased from 10mg/kg daily to 10 mg/kg either 8 or 12 hourly in the BNFC [173] Table 1-11. However, this increase was not founded on neonatal PKPD data. At present there are neonatal peak and trough data whereas the evidence suggests that AUC/MIC ratios are the best predictors of cure [115, 185]. To provide neonatal data with AUC additional sampling time points to peak and trough are required. Aggarwal *et al.* [185] compared the peak and trough ciprofloxacin concentrations in 24 neonates administered 20mg/kg/day on days 1, 3 and 7, the mean peak ranged from 2.3 to 3.0 µg/mL and trough 0.7 to 1.0 µg/mL. They found no difference in concentrations for VLBW and non VLBW babies. Premature babies may have higher or lower drug concentration or AUC as has been shown with gentamicin [126].

In both recruiting sites ciprofloxacin is part of the standard clinical protocol for neonatal sepsis (Table 1-12). Liverpool Women's NHS Foundation Trust Neonatal Unit admits over 1000 newborn babies a year of whom approximately 100 are prescribed ciprofloxacin as a second line treatment for suspected sepsis. Approximately 300-400 babies a year are treated for suspected late onset sepsis. Ciprofloxacin was introduced in this neonatal unit to minimise pressures towards cephalosporin resistance 20 years ago [183]. Alder Hey NHS Foundation Trust is a lead centre with over 20 paediatric wards including a Neonatal Surgical Unit and Paediatric Intensive Care Unit (PICU). The PICU has 23 beds and admits over 1000 children a year of which 48.1% are aged less than one year and of these 58.4% less than three months of age [186].

Table 1-11 British National Formulary for Children (BNF C) Ciprofloxacin Regimens

| Indication | Intravenous dose (mg/kg) | |
|---|---|---|
| | Neonate | Infant >1 month |
| Complicated urinary tract infection | 6 (12 hourly) | 6 (8 hourly) |
| Severe respiratory tract infections (RTI) | 10 (12 hourly) | 10 (8 hourly) |
| Pseudomonal lower RTI | | 10 (8 hourly) |
| Anthrax | | 10 (12 hourly) |

Table 1-12 Recruiting Sites Intravenous Antibiotic Guidelines

| | Indication | Antibiotic | Dose (mg/kg) | Frequency (hours) |
|---|---|---|---|---|
| **Liverpool Women's NHS FT** | **First Line:**<br>Early Onset (<5 days birth) | Benzylpenicillin | 25 | 12 |
| | | Gentamicin | 4.5 | 36 |
| | Late Onset (>5 days birth) | Co-amoxiclav | 30 | 8 |
| | | Gentamicin | 4.5 | 36 |
| | **Second line**<br>Renal impairment<br>Lack of clinical response<br>Sensitivities of organism | Ciprofloxacin<br>(add or replaces Gentamicin) | 10 *<br>*(increased from 5 in 2011)* | 8 or 12 |
| | Meningitis | Cefotaxime | 50 | 8 |
| | CoNS | Teicoplanin | Load:16<br>then 8 | 24 |
| Alder Hey Children's NHS FT<br>(No standard protocol) | Severe Infection<br>Hospital acquired<br>Central line | Ciprofloxacin | 10 | 12 (Neonate)<br>8 (Term) |
| | | | | |

Table 1-13 Ciprofloxacin PK studies in children and neonates

| **Author** | **Year** | **Age Group** | **N=** | **Dose mg/kg/ day** | **AUC (SD)** | **MIC range mg/L** | **Serum $C_{max}$ mg/L (SD)** | **Serum $C_{min}$ mg/L** | **V l/kg (SD)** | **Cl L/h/kg (SD)** | **t ½ (SD)** | **CSF mg/l** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Isaacs *et al.* [182] | 1986 | Preterm 26 weeks PMA | 1 (CSF 1) | 4 - 6 | N/A | 0.25 – 1 | 0.98 to 1.3 | 0.11 - 0.29 | | | | 0.10 – 0.37 (11- 28% of serum peak) |
| Bannon *et al.* [183] | 1989 | Preterm 24 -29 weeks PMA | 6 (CSF 1) | 10 | - | 0.03 – 0.06 | 1.45 – 5.7 | 3rd dose: 0.04-2.6 | | | | 0.88 - 1.45 64% of serum peak (mean) |
| Lipman *et al.* [115] | 2002 | Infants 3-12 months | 10 | 20 | 32.60 $_{0\text{-}24}$ (2.88) | N/A | 6.97 (1.48) | 0.19 (2.34) | 1.87 (1.39) | 0.58 (1.46) | 3.74 (1.58) | |
| | | Children 1-5 years | 10 | 20 | 31.4 $_{0\text{-}24}$ (2.84) | N/A | 7.18 (1.42) | 0.15 (2.17) | 1.54 (1.20) | 0.56 (1.33) | 2.93 (1.26) | |
| Peltola [187] | 1992 | Term 5 – 14 weeks | 7 | **Oral** 15 mg/kg | 16.1 $_{0\text{-}\infty}$ (7.4) | N/A | 3.3 (1.3) | | N/A | | 2.73 (0.28) | |
| | | Children 1-5 years | 9 | | 5.3 $_{0\text{-}\infty}$ (3.3) | | 2.1 (1.7) | | | | 1.28 (0.52) | |
| Aggarwal *et al.* [185] | 2004 | Preterm 32 weeks PMA | 24 | 20 | N/A | N/A | 2.6 (0.4) | 0.83 (0.16) | | | | |

#### 1.2.2.1 Pharmacovigilance

Too little exposure to an antimicrobial leads to an ineffective drug regimens, and too much creates the potential for adverse effects [72]. Critically ill babies have complex clinical conditions; adverse events may be confounded by events not related to microbiology or antimicrobial therapy but associated with the underlying clinical condition including congenital abnormalities, other interventions or concomitant medication.

In a critically ill population the choice of drug therapy balances the risk of adverse reactions with the high risk of morbidity and mortality from Gram-negative septicaemia. Mortality in neonates increases from 18% to 36% when Gram-negative organisms are confirmed in blood cultures [18]. The potential risk of an adverse event (such as arthralgia that is likely to resolve) needs to be proportionate to the risk of the outcome of Gram-negative sepsis which has high mortality. The observed effect of the drug may have a similar effect to the clinical condition on organ function [188]. It is difficult to infer causality in this population without a blinded randomised controlled trial. A large prospective observational cohort study of adverse drug reactions in children (n= 6,601) admitted to hospital deliberately excluded PICU patients because the causality assessment was more difficult and requires different methodologies for detection [189]. A reaction may manifest weeks or more after therapy such as ototoxicity [63]. Neonates are more susceptible to drug related developmental disorders and adverse drug reactions that can sometimes only be detected later in life [79].

Previously reported adverse reactions for ciprofloxacin are summarised in the MHRA Public Assessment Report [53] Table 1-14. A systematic review of ciprofloxacin in paediatrics reported 105 articles that met the inclusion criteria involving 16,184 patients. A total of 1,065 adverse events were reported (risk 7%, 95% CI 3.2% to

14.0%) of which the most frequent were musculoskeletal, abnormal liver function tests, nausea, changes in white blood cell counts and vomiting [190]. This review did not include reports of seizures but Lipman *et al.* [115] found seizures with higher Cmax. This review did not provide a separate analysis of blinded randomised controlled trials and many of the reported adverse events are commonly reported clinical symptoms in sepsis.

Table 1-14 Expected Adverse Reactions for Ciprofloxacin

| **Body System** | **Adverse reaction/Hazard** | **Likelihood** Rare, Low, Medium or High |
|---|---|---|
| Joints | Arthropathy/ tendonopathy | M |
| Vein | Phlebitis | L |
| Liver Function | Failure /Pancreatitis | L |
| Renal Function | Failure /Crystalluria | L |
| Blood cells | Deranged | L |
| Gastro-intestinal | Colitis if severe and persistent *Clostridium Difficile* | M |
| Anaphylaxis /Allergy | Shock | R |
| Neurological | Convulsions | M |
| Cardiac | Ventricular arrhythmias /Prolonged QT interval | |
| Skin | Rash/ Photosensitivity | M |
| Syndromes | Stevens Johnson /Lyell | R |

Reproduced from the Summary of Product Characteristics and MHR Public Assessment Report [53]

The potential risk of arthropathy has deterred clinicians from prescribing ciprofloxacin to children. An early report of arthropathy for a child led to further safety studies in juvenile laboratory animals exposed to quinolones (nalidixic, oxolinic and pipemidic acids) [191, 192]. Studies with mature animals (rats and dogs) revealed no evidence of cartilage lesions whereas in young beagle dogs (4-12 months of age) severe articular changes were observed at therapeutic doses following two weeks of treatment and after a period of five months [53, 191]. The long term effects on growth were studied in a retrospective cohort of 205 premature babies (61 cases and 144 controls) and found no evidence of change to their linear growth at 12 months

corrected age [193]. The systematic review of adverse events described above found 258 musculoskeletal events occurred in 232 paediatric patients (risk 1.6%, 95% CI 0.9% to 2.6%) [190]. Pooled safety data of controlled trials in this review estimated the risk of arthropathy as 1.57 (OR 95% CI 1.26 to 1.97). The majority of musculoskeletal events (86%) were classified as possibly related to ciprofloxacin and a re-challenge was only done in eight patients of which only one was positive [194]. The review concluded that musculoskeletal adverse events are reversible following withdrawal of the ciprofloxacin with further symptomatic management [190]. The WHO Subcommittee on Essential Medicines for Children found arthropathy was not convincingly correlated with the use of fluoroquinolones in children [195].

Many of the adverse events that have been reported are common in sepsis. Joint pain is a common presentation in children and has been associated with as many as 100 different diagnoses besides traumatic aetiology [196]. The hips of newborn babies may be affected by a number of other conditions including developmental dysplasia or infection. McCarthy *et al.* categorised the cause of joint complaints in 250 children, 19.6% related to bacterial infection, 18% orthopaedic, 17.6% autoimmune disorders, 44% miscellaneous. Autoimmune or infectious disorders were eight times more likely to present with joint pain if the temperature was > 38°C or elevated erythrocyte sedimentation rate ≥ 30mm/h [197].

The clinical assessment of hips in neonates is challenging as they are not weight bearing and non-verbal. There are difficulties in assessing whether there is a painful response to handling or startle reflex. Due to the limitations of diagnosis in non-weight bearing children there were no reports of arthropathy in children under six months included in the review, the youngest in the review was seven months [198]. MRI provides a useful tool to assess the hips in adults [199]. Pradhan *et al.* (1995) assessed the MRI scans of children following ciprofloxacin between day 10 and 15 of therapy and found no significant cartilage damage [200]. The normal MRI

appearances of the neonatal hip have been described in detail for neonates [201]. MRI assessment of arthralgia requires the development of a method to define and validate biomarkers relating to joint spaces and cartilage thickness. The Liverpool Archive of MRI in Babies (LAMB study) is a registry of MRI that commenced in 2011 to compare the liver, hip and brain of healthy babies with clinically indicated scans. Few are treated with ciprofloxacin therefore conclusive results will take several years. Altered hepatic or biliary function is common in sepsis. Sepsis associated cholestasis induced by inflammation is a common complication in patients with extra-hepatic infection or inflammatory processes [202]. This may be associated with the functional immaturity of immune system and bile formation especially in the first weeks of life. Endotoxins from Gram-negative bacteria can inhibit the function of the membrane transporter in hepatocyte cells, thus leading to cholestasis [203]. Oswari *et al.* [202] found elevations of serum AST were a marker of multi-organ dysfunction that reflects the severity of sepsis. Victor *et al.* found extremely premature infants had high plasma enzyme activities when compared to babies at a later corrected gestational age that may be due to more severe illness immediately after birth [204].

Ciprofloxacin has been associated with seizures in adults and children with a higher Cmax [53, 115]. Ciprofloxacin is active in the central nervous system, and sometimes causes psychotic symptoms as side effects, known to be partly mediated by inhibition of the GABA A receptor [198]. Adverse reactions of the central nervous system are well-known complications of quinolone therapy but the mechanism is unknown [205]. Pre-clinical studies suggest the effects are less in ciprofloxacin than other quinolones and may be linked to magnesium levels [206].

Neonatal venous access is limited and cannulation is more difficult than in adults due to the small size of veins. Therefore it is particularly important to protect against phlebitis when administering a drug. The pH of ciprofloxacin is 3.4-4.5. The drug is administered slowly over 60 minutes. An injection site reaction is described as an

uncommon reaction in the SmPC (ranges between >1 in 1,000 and <1 in 100 patients) [53]. The Visual Infusion Phlebitis Scale [207] is a method for monitoring infusion sites recommended by the Infusions Standards Groups within the Royal College of Nursing (UK) [208] (validation is unpublished but cited by Gallant [209]).

The TINN Consortium (Work Package 2) undertook preclinical studies in mice to determine neuro-developmental and physiological parameters including vital signs, blood gases. Arthrotoxicity was not identified (unpublished TINN Interim Report June 2011). Preclinical safety data for adults revealed no special hazards for humans based on conventional studies of single dose toxicity, repeated dose toxicity, carcinogenic potential or toxicity to reproduction [53]. A review of safety in relation to quinolones recognised the limitations of adverse event reporting and the risk of reporting non drug related events. The most reliable comparative data regarding tolerability is from double blind randomised controlled trials [205]. In a double blind trial Pichler *et al.* compared adults administered ciprofloxacin and a placebo and found the same rate of adverse events [210]. This illustrates the need for a blinded RCT when evaluating adverse events and adverse reactions in relation to drug administration particularly in critical illness.

## 1.3 Pharmacogenomics

The host response to infection is at least in part influenced by genetics as susceptibility to sepsis and the clinical course of patients is highly heterogeneous [211]. Individual differences in efficacy and in elimination might partly depend on genetic polymorphisms are changes in DNA sequence of active drug transporters [57, 121]. These polymorphisms might affect the likelihood of individual adverse reactions, toxicity and efficacy of drugs. Since ciprofloxacin is not extensively metabolized by enzymes a genetic polymorphism of the metabolising enzymes are less likely to have any influence. Drug metabolizing enzymes and transporters that contribute to the individual and age dependent capacity to metabolize and eliminate drugs are genetically determined and some individuals show an exaggerated response to the drug [119]. The main genetic influence may be associated with elimination and distribution associated with active drug transporters as ciprofloxacin is transported by organic anion transporter 3 (OAT3) [121].

To date there have not been any prospective clinical trials to evaluate whether a patient's genotypic profile improved efficacy, reduced adverse reactions, or lowered the overall costs of therapy [72, 212]. Vast population studies are required to evaluate the influence of specific polymorphisms. An understanding of how genetic patterns vary according to outcome will allow investigators to stratify biomarkers and develop novel pathways for therapeutic targeting [213]. DNA samples collected during the PK study may in future contribute to individualised antimicrobial therapy.

### 1.3.1 DNA sampling in neonates

In this neonatal unit DNA samples are obtained from buccal cells to minimise avoidable blood loss. This method may not provide sufficient quantity and quality of DNA for pharmacogenomic analysis and blood scavenged from clinical samples that would otherwise be discarded could supplement the quantity of DNA required. There is a theoretical risk that blood from recently transfused babies may be contaminated by the donor's DNA either in white cells or free circulating DNA.

Transfusion Associated Microchimerism (TA-MC) describes the presence of leucocytes in a recipient from the donor following a transfusion [214, 215]. Critically ill neonates may be at increased risk of TA-MC as frequent clinical sampling can result in multiple transfusions. Immediately following transfusion the concentration of donor leukocytes has been reported to increase transiently for 3-5 days [216]. As a precaution some clinical laboratories recommend delaying sampling for one week following transfusion [217]. Other studies suggest TA-MC can persist for years and be as much as 5% of circulating leucocytes [214, 215].

The risk of microchimerism may have reduced since 1996 as blood products are leukocyte depleted to $<1 \times 10^6$ per unit based on the NHS Blood and Transplant Service standards Regulation SI 2005 No 50 [218]. In theory, this may reduce the risk of TA-MC allowing DNA to be scavenged from discarded clinical samples. Further reduction of white cells in blood products may be achieved by gamma irradiation or methylene blue however free circulating DNA may still be present.

When DNA has been extracted and amplified by Multiplex Polymerse Chain Reaction (PCR) (sensitivities to concentrations <0.5 ng of DNA) the DNA 'fingerprint' is illustrated on an electropherogram to compare the buccal cell DNA with the blood derived DNA from the same patient A DNA profile usually has two alleles (one from each parent) but when more are present this suggests either i) contamination of non-

self cells or ii) artefacts and stutters that are commonly observed in the electropherograms. The Genemapper analysis software provides an electropherogram to illustrate the allelic ladder with sixteen highly variable loci (alleles) known to have a high degree of inter individual variation. In terms of powerplex a homozygote is when both alleles are the same and heterozygote is when alleles differ and can be resolved from one another. Artefacts may appear as one or two repeat units after the true allele peak whereas a stutter is more likely to appear before the true peak [219]. This provides a rapid, non-radioactive method designed to obtain a DNA type, or genetic fingerprint suitable for very small amounts of DNA.

Contamination by a blood donor would result in many different peaks particularly as the 16 alleles selected for comparison have a high degree of individual variability. In contrast, for example foetal DNA can be contaminated by the mother's after birth, in this situation a comparison is required for the true peaks in each allele as the mother and foetus may have the same alleles genetically [220, 221]. The mother's genotype is presented on an electropherogram then the mother's alleles are identified on the fetal sample. The foetal alleles are tallest as this relates to the amount of material present, contaminating maternal alleles are much smaller and where the mother and foetus share alleles a larger than normal peak is obtained.

Figure 1-27 shows a typical electropherogram obtained from the DNA 'fingerprinting' from a fetal sample with maternal contamination (for example from chorionic villus sampling). To allow the maternal contaminating genotype to be identified with certainty from the electropherograms a separate DNA sample from the parents is mapped. The labels in blue boxes 'M' show the maternal alleles, the labels in red boxes 'F' show the foetal alleles, and the green labels show shared maternal and foetal alleles. http://www.softgenetics.com/chimerMarker_9.htmL (accessed 19-3-2013).

Table 1-15 Leukocyte depletion of blood products

| **Blood Product** | **Leukocyte depletion Mean 106/Unit* (SD)** | | **UK Standard SI 2005 No 50** |
|---|---|---|---|
| Red Packed Blood Cells SAGM | 0.30 | 0.29 | <1 x $10^6$ / unit |
| Platelets | | | |
| Apheresis | 0.84 | 19.32 | <1 x $10^6$ / unit |
| Pooled | 0.33 | 0.28 | |

The NHS Blood and Transplant Service standards for leukocyte depletion are set by the Blood Safety and Quality Regulations [222] . The UK standard and the level of leukocyte depletion achieved are from personal communication NHS Blood Transfusion Service email date 3/2013.

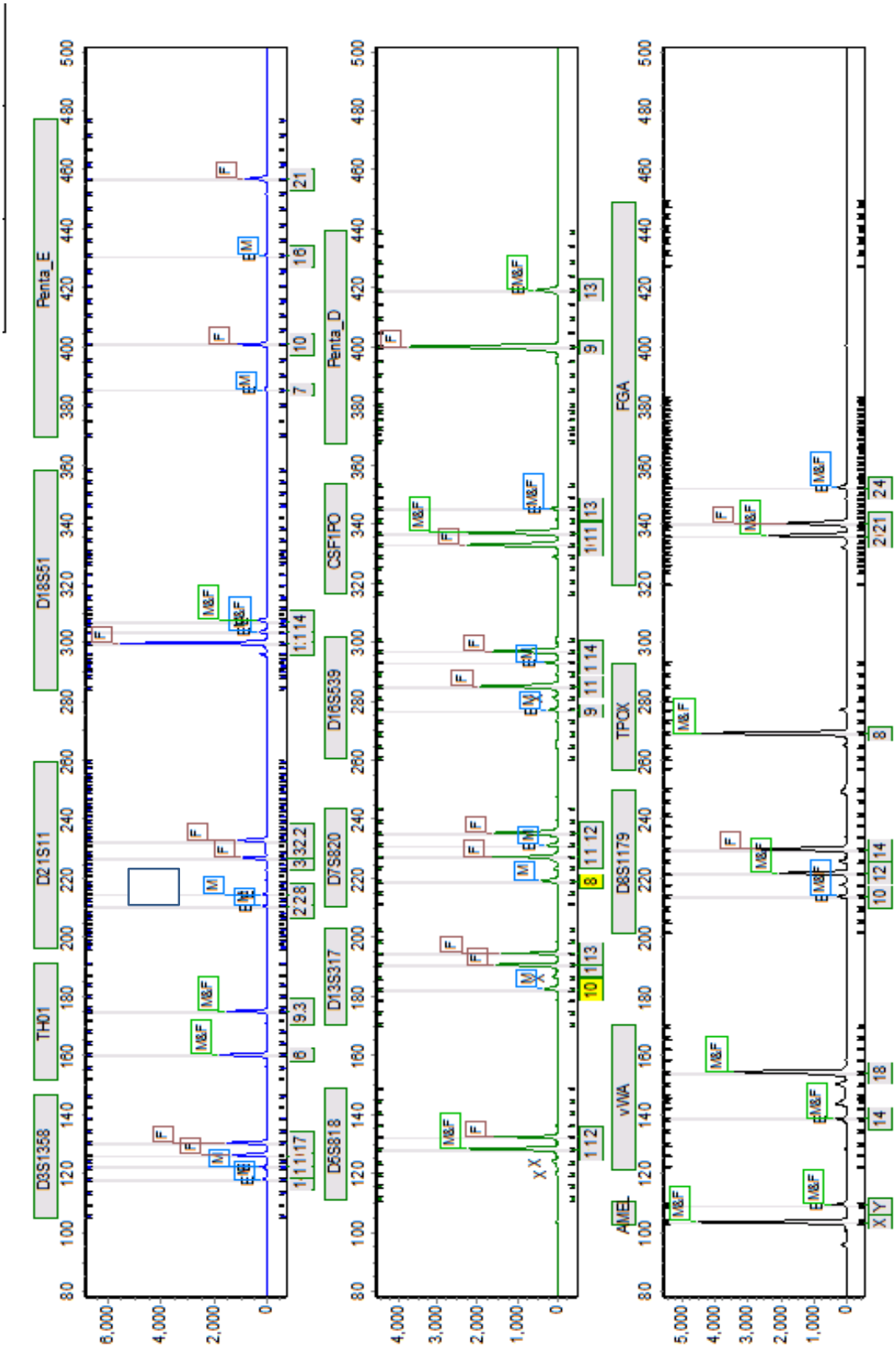



Figure 1-27 Foetal DNA with maternal contamination

This electropherogram was representative of when both the mother and the foetus' alleles are known.

### 1.4 Rationale for evaluating the PK PD of Ciprofloxacin

Successful drug therapy is directly dependent on the administration of the optimal dose at the ideal dosing interval and therefore requires PK / PD and safety data specific to the population [223, 224]. In addition the increasing burden of resistance and lack of new antibiotics for Gram-negative bacteria leads support the need to optimize existing antimicrobials [6].

At present ciprofloxacin is frequently administered throughout Europe but not authorised for use in neonates or infants below three months age. The TINN Consortium conducted a survey of the prescribing practices in Europe for ciprofloxacin in neonatal units (n=193). Ciprofloxacin was prescribed at 25% of these units (n=50) [184] Table 1-10. In the USA annual ciprofloxacin prescriptions for patients younger than 18 years is estimated as 150 000, 20% <1 year age [225]. Off label prescribing has resulted in wide variation in regimen internationally the optimal regimen is unknown yet it is commonly prescribed to neonates [173].

Table 1-16 European survey of Neonatal Units Indication for Ciprofloxacin (n=50)

| Indication | Units N=50 | % |
|---|---|---|
| **First line therapy** | | |
| Neonatal Sepsis | 1 | (2) |
| Severe sepsis | 3 | (6) |
| CNS documented infection: (meningitis, meningoencephalitis, ventriculitis, cerebral abscess) | 13 | (26) |
| Suspected multi-drug resistant strain infection | 15 | (30) |
| **Second Line Therapy** | | |
| Sepsis resistant to first line empirical therapy (other than ciprofloxacin) | 7 | (14) |
| Severe sepsis resistant to first line empirical antibiotic therapy | 17 | (34) |
| Culture-proven sepsis due to multi-drug resistant organisms but sensitive to ciprofloxacin | 41 | (82) |

Ciprofloxacin is the only fluoroquinolone on the WHO Essential Medicines for Children list. The WHO Expert Subcommittee concluded that sufficient evidence is available to support the use of ciprofloxacin as a second-line treatment for specific, severe infections in paediatric patients [226].

The TINN consortium elected to conduct were funded by the EU Framework 7 Grant to develop a Paediatric Investigational Plan [73] in order to improve prescribing information about ciprofloxacin.

## 1.5 Neonatal Drug Development

### 1.5.1 European Medicines Agency Regulations and Guidance

EMA require a Paediatric Investigation Plan (PIP) describing the strategy for developing the medicinal product [80]. To fulfil the requirements of a PIP the European TINN Consortium prepared the following work packages:

- Pre-clinical in silico experiments
- European survey of prescribing ciprofloxacin for neonates.
- Systematic review of adverse events
- Established an Ethics and Safety Advisory Board (EAB)
- Pharmacogenomics
- Pharmacokinetics and short term safety reporting of ciprofloxacin

The EU Paediatric Regulation aims to improve medicines for children by licensing medicines specific to each age group [73].The EMA provide specific guidance for clinical trials involving pre-term and term newborn infants relevant to drug development [79]. Covariates for premature babies should include post-natal age, postmenstrual age and gestational age. Over this dynamic period of development rapid changes can occur in body weight, surface area and body composition and additional categories may be required for babies ‘small for gestational age’, and ‘low birth weight’. Post-natal weight loss may be 10% of birth weight and body weight in preterm neonates may increase three fold during post-natal medical care requiring doses to be recalculated. Recruitment should represent nephrogenesis before and after 34 weeks of PMA then stratify to further sub-ages for those <26 weeks, 26-29 weeks, 30-33 weeks, 34-36 weeks, 37-40 weeks and > 40 weeks gestational age. Known complications including respiratory distress syndrome, patent ductus

arteriosus, pulmonary hypertension, intra-ventricular haemorrhage, necrotising enterocolitis, retinopathy of prematurity and bronchopulmonary dysplasia should be evaluated as secondary endpoints. Other important factors include renal and hepatic clearance, concomitant medications, serum-bilirubin, feeding patterns, protein binding, displacement issues, especially bilirubin, and penetration of medicinal products into the central nervous system [79].

The EMA drug development guidelines for antimicrobials require efficacy studies in relation to different pathogens including multi-drug resistant, even if underpowered [63]. A non-clinical assessment of anti-bacterial activity should document *in vitro* bactericidal activity of the test antibacterial against recent clinical isolates for a period of five years according to EUCAST. Ideally several hundred isolates should be tested from different countries relevant to the clinical indication with a minimum of ten isolates for rare organisms. [227]. Selecting a drug regimen should be informed by PK/PD data from the population treated rather than healthy subjects. The PK-PD analysis should include the highest MIC distribution for wild type populations of pathogens (non-resistant) treatable with well tolerated dose regimens [63].

The EMA guidance in relation to antimicrobials for paediatrics and neonates are limited as the guidance is general to drug development [79, 80]. The EMA provide a guideline on the evaluation of medicinal products indicated for treatment of bacterial infections which includes a brief paragraph on children [63]. There are specific challenges that need to be addressed regarding antibiotic trials in neonates and children. When the TINN consortium submitted a PIP application, the EMA requested a randomised controlled trial to be carried out to determine safety and efficacy. To power a paediatric sepsis trial using a baseline mortality of 10% as a primary end point would require 4000 babies to detect a significant relative reduction in the risk of mortality [29]. Due to the very small number of neonates with confirmed Gram-negative sepsis that are eligible for such a trial, it was estimated this could take a

decade. Accordingly, the requirements of the EMA Paediatric Committee were not practicable in this patient population. Similarly, it was agreed in the protocol that safety data would be tabulated to provide a description of adverse events during treatment with ciprofloxacin. In terms of safety the confounding variables associated with prematurity and critical illness would require a randomised controlled trial for a definitive evaluation of safety outcomes [228]. Further analysis was not planned in this trial due to the complexity of determining causality of adverse reactions in critical illness [228]. Similarly, other studies of adverse drug reactions have excluded critical illness for this reason [229, 230].

#### 1.5.1.1 Clinical Outcome Measures for Neonatal Suspected Sepsis

To evaluate safety or effectiveness requires a definition of the optimal outcome. At present there are no core outcome sets specific to neonatal sepsis [29, 220, 221]. There are limitations to using mortality as an endpoint due to the high number of neonates required when the incidence of both death and Gram-negative blood cultures are generally very low in children [29]. Composite outcomes such as organ failure–free days or ventilator free days are an alternative in critical illness [29, 220, 221]. The advantage of selecting a short term outcome may determine a temporal association between the antibiotic and the outcome. Composite measures such as 30 day mortality or ventilator free days are confounded by death and morbidity from other causes in critically ill infants [231]. Therefore biomarkers such as C-reactive protein or safety parameters such as liver and renal function tests are used in clinical practice [221].

### 1.5.2 Regulatory barriers to neonatal clinical trials

To protect vulnerable groups including children the EU Clinical Trial Directive 2001/20/EC (2001) includes additional regulatory and ethical principles [73, 232, 233]. PK Clinical Trials are classed as Clinical Trials of Investigational Medicinal Products (CTIMPS) and regulated by the MHRA [234]. The ethical concern for including

children in research is balanced against the risk of administering medicinal products to a population in which they have not been appropriately tested to ensure safe and effective drug administration [73, 235]. Inadequate dosage information has led to increased risks of adverse reactions including death and ineffective treatment through under dosing and toxicity with overdosing [236]. The EU Paediatric Regulations were introduced to provide incentives for medicines research for this population [73]. More recently, the Academy of Medical Science Report 2012 claimed the clinical trial regulations were a barrier to research and the Department of Health (DOH) directed the MHRA to establish a more proportionate approach based on the risk of the investigational medicinal product (IMP) [232]. A joint project in the UK by the MRC, DOH, MHRA and in Europe led by the MHRA aimed to develop a risk adapted approach that classify the risk of a trial as Type A, B or C relative to the drugs marketing authorisation or use in standard care [232, 237]. Within this proposal the MHRA recognised that paediatric medicines are often prescribed off label even though they are prescribed in standard care [232]. To determine whether the risks could be mitigated the DOH directed the MHRA to publish a model and requested a model for the neonatal PK clinical trial for an off label medicine.

The ethical challenges of paediatric research were extensively considered by the European Union *ad Hoc* group who provided guidance in respect of blood volume, parental consent, distress, confidentiality, trial design and data reliability [238]. Methods to minimise the volume of blood the distress of sampling procedures and development of assays using small volumes are essential particularly when the drug level provides no direct benefit to the patient [135]. To protect neonates the EMA guidelines specify the volume of blood that can be collected for the purpose of research [79]. The Royal College of Paediatrics report categorised blood sampling as a low risk relative to other interventions [238].

Ciprofloxacin is prescribed for standard care rather than for the purpose of research the risk to the patient is mainly due to the additional blood sampling. Parental consent is required at a stressful time. To ensure the rights, safety and wellbeing of trial subjects and be legally 'valid' consent for research must be provided 'voluntarily' by a person who has been fully 'informed' who has the 'capacity' at the time it is requested to make the decision [233, 239]. Parents may not be available when ciprofloxacin is prescribed at any time of the day or night and samples are required within minutes of the first dose. Emergency deferred consent was introduced in 2006 for children in specific situations when treatment is required urgently to be recruited if approved by an Ethics Committee [240]. This allows consent to be deferred to a more appropriate time for the families. This research has an ethical duty to ensure that the data generated are reliable. The precision of sampling and drug administration are crucial to PK analysis.

### 1.5.3 Summary

Antibiotics are central to the management of neonatal infection. The principles of antibiotic development are well understood but have not been applied to neonates or young infants, as a result there are no antimicrobials authorised by EMA specifically for sepsis in children [27]. There is an unmet therapeutic need for the paediatric population. Children have specific PK characteristics which have prompted European legislation [73]. As a consequence of this legislation the TINN consortium was funded to study ciprofloxacin. This thesis aims to support prescribing decisions relating to ciprofloxacin in neonates and young infants.

Following discussions with EMA a PK clinical trial was designed. As paediatric PopPK trials are conducted in patients with the disease the data better reflects clinical use [80]. A prospective PK clinical trial provides data on PK parameters and the variability between each gestational sub-age group for premature and term infants representing every four weeks. Short term safety data was monitored systematically consistent with

Regulatory requirements. This is the first detailed PK model with AUC data of ciprofloxacin in neonates. PK clinical trials in neonates are complicated by rapid physiological changes during sepsis, disease influence on organs and technology used for organ support and therefore need to consider many covariates [78, 83, 241]. The maturity of renal function and metabolic pathways can have profound implications on drug disposition in infants [32, 242]. Sufficient eligible babies are required to represent each of the six sub age groups of neonates [79, 80]. There is a lack of *A priori* data to inform the sampling schedule as this cannot be reliably extrapolated from adult, adolescent or even older children for this population. Changes in total body water including dehydration or fluid resuscitation and interventions such as ventilation, concomitant medications, haemodynamic effects of congenital cardiac defects or inotropes and infection may influence PK/PD [79, 96, 223]. Data reliability is a considerable challenge due to the precision required when administering minute drug volumes, complex infusions and limitations to blood sampling in a clinical trial. Data reliability is essential as PK studies are rare in neonates and these data may inform prescribing globally. Parental consent is required at a stressful time when families are dealing with the birth of a premature baby diagnosed with suspected sepsis.

In critical illness the reporting of clinical outcomes and safety are influenced by complex covariates. There are few high quality studies due to the small number of sepsis patients and difficulties in assessing outcomes [80]. To address this, a retrospective study reflects on the microbiological and clinical outcome of neonates with Gram-negative sepsis treated with ciprofloxacin over a six year period. Gram-negative organisms were re-cultured to assess MIC creep and determine the MIC of ciprofloxacin for comparison with the neonate's clinical outcome. Clinical breakpoints used to predict therapeutic success of antimicrobial therapy are currently informed by adult dose regimens and outcome data.

The challenges of neonatal clinical trials associated with the regulatory framework have been recognised by the DOH following the Academy of Medical Science Report [232, 235]. In response the MHRA were required to develop a risk proportionate model for PK clinical trials in neonates. As part of this thesis a model for neonatal pharmacokinetic clinical trials was developed and published by the MHRA demonstrating methods to mitigate risks within a proportionate regulatory framework.

### 1.5.4 Primary Aim and Objectives

**Primary Aim:**

**To evaluate the pharmacokinetics, microbiological and clinical outcome of neonates and infants with late onset Gram-negative sepsis treated with ciprofloxacin.**

**Primary Objectives:**

- Describe the population PK of ciprofloxacin in neonates and young infants
- To optimize the method of collecting DNA samples whilst minimising blood loss from neonates for future pharmacogenomic analysis.
- To describe the microbiological and clinical aspects of the use of ciprofloxacin on a Neonatal Intensive Care Unit through a retrospective cohort study.
- To develop a proportionate regulatory model for neonatal pharmacokinetic trials that reduces regulatory barriers whilst mitigating potential risks to the safety of neonates and the reliability of trial data for the MHRA.

# Chapter 2 Pharmacokinetic Clinical Trial

## 2.1 Overview

This Chapter describes a population pharmacokinetic clinical trial of ciprofloxacin administered to preterm/term neonates and young infants up to 3 months of age with suspected Gram-negative sepsis.

This clinical trial was developed and conducted at a neonatal lead centre and paediatric lead centre in the UK over an 18 month period between 2011 and 2012. The protocol was developed consistent with the UK Regulatory and Ethical approvals. A clinical trial culture for PK design was developed to ensure data reliability and optimise recruitment. Trial methods including standard operating procedures and trial tools were developed consistent with the local practice at sites. The challenges of PK trials in critically ill children including clinical, ethical and regulatory were explored and solutions developed. The clinical interpretation of the data was explored to ensure data captured included the developmental, interventional and pathophysiological factors that influence the parameters for each sub-age group between 24 and 48 weeks PMA. The clinical trial protocol, case report form, standard operating procedures were developed then the trial was conducted by the research fellow as part of this thesis.

The PK model building using NONMEM was completed by the PK analyst appointed to the consortium. This includes Figures 2.18 to 2.21 and Tables 2.14, 2.15 and 2.18.

### 2.1.1 Aims and Objectives:

**Aim:** Describe the population PK of ciprofloxacin in neonates and young infants

**Primary Objective:**

- To evaluate the multiple-dose PK of ciprofloxacin in neonates and young infants (24–52 weeks postmenstrual age) with suspected or proven Gram-negative infection.

**Secondary Objectives:**

- Covariate analysis including postmenstrual age, gestational age, postnatal age, weight and serum creatinine.
- PK parameters including apparent volume of distribution and half-life.
- Describe the demographics of the population and outcome of treatment episodes
- Determine the extent to which ciprofloxacin penetrates the cerebrospinal fluid in neonates.
- Describe the short-term safety profile of ciprofloxacin
- Evaluate the tolerability of ciprofloxacin.
- Describe the short term clinical outcomes C-reactive protein and mortality.

**Primary Outcome Measures**

- Ciprofloxacin plasma concentration and population PK parameters [maximum concentration, clearance, area under the curve (0-tau)], their relationship with covariates and inter-individual variability (CV%).
- PK variables include apparent volume of distribution and half-life.

**Secondary outcome measures:**

- CSF concentration relative to the time of the dose
- Adverse events (AEs) and serious adverse events (SAEs).
- To evaluate the tolerability indicated by withdrawal of ciprofloxacin.
- Clinical outcome of treatment (C–reactive protein and mortality).
- Pre and post treatment MIC and PCR of organisms in faeces to assess rapid resistance

## 2.2 Methods

### 2.2.1 Study design:

This was a prospective, open label, population PK clinical trial of ciprofloxacin with sparse sampling at informative time points with additional scavenged samples. Ciprofloxacin was administered as part of standard care for suspected or confirmed sepsis. The clinical trial protocol and standard operating procedures defined the methods and tasks including:

- Recruitment and screening
- Drug prescribing and intravenous administration
- PK samples at informative time points and scavenged blood sampling
- Laboratory processing
- Sample collection, shipping and storage
- Safety monitoring and risk assessment
- Data management

#### 2.2.1.1 Recruiting sites:

There were two recruiting sites: Liverpool Women's NHS Foundation Trust (Neonatal) and Alder Hey Children's NHS Foundation Trust (Paediatric). Both are lead tertiary centres with facilities for critical care. The Neonatal Unit has 1,200 admissions per annum and the Paediatric Intensive Care Unit 1,100 per annum. In addition there are 23 wards at the children's hospital. Non–recruiting sites were set up at 40 hospitals to collect follow up data on adverse events and post treatment faeces samples. This required R&D approvals, a Principal Investigator with GCP and trial training for pharmacovigilance reporting.

#### 2.2.1.2 Patient population – Inclusion and Exclusion

**Inclusion criteria**

- A clinical decision to prescribe ciprofloxacin according to the unit's clinical protocol for managing late onset sepsis.
- 24 – 52 weeks post menstrual age (calculated from the first day of the last menstrual period)
- A parent or person with parental responsibility gave consent.
- Ciprofloxacin commenced within <48 hours of the first PK sample

**Exclusion criteria**

- Ciprofloxacin commenced before the fifth day of life
- Unlikely to survive more than 48 hours as judged by the attending clinician.
- Active cooling, haemofiltration, extra-corporal membrane oxygenation or peritoneal dialysis.
- Recruited to other trials if the Investigators deemed that one trial would interfere with the validity of the other or if additional blood samples would exceed the recommended blood volume guidelines in the protocol.

Pre-trial feasibility data was based on pharmacy records for the centralised sterile supplies unit and the neonatal BADGER patient data system. This indicated the majority of babies administered ciprofloxacin were located in the PICU or NICU and high dependency wards when diagnosed with suspected Gram-negative infection.

#### 2.2.1.3 Sample size

The target recruitment was 50 babies stratified to represent every four week development between 24 weeks and 52 weeks PMA (Table 2-1) consistent with the EMA guidelines [79, 80]. The target recruitment was five to eight patients per four week period.

Table 2-1 Target recruitment stratified for seven sub-age groups

| Sub-age groups - Post Menstrual Age (weeks) | |
|---|---|
| **Pre–term** | **Term Infants** |
| **24-27** | **40-43** |
| **28-31** | **44-47** |
| **32-35** | **48-52** |
| **36-39** | |

2.2.1.4 Sparse blood sampling schedule and scavenged samples

Three blood samples per day were collected at the start of treatment on the first or second day, then a further three at the end of treatment on day five, six or seven. In preterm neonates weighing <1000g the sample volume was reduced from three to two samples per day to remain within the recommended volume limit. Depending on whether the dose regimens was prescribed either eight or twelve hourly they were allocated to one of four sampling schedules Group A, B, C or D Table 2-2. The peak drug concentration was collected within three minutes of the end of the drug infusion T1. All other concentrations were taken within ten minutes of set time points ranging from T2, T3, T4, T6, T8 and T12 calculated as hours after the infusion started. These time points were not informed by prior data as this is not available but were distributed over twelve hours with more frequent samples in the early period. The researcher selected the sampling group in discussion with the clinical team to coincide when possible with clinical samples.

A minimum of 0.2 mL blood was required for analysis to provide between 30 µL and 150 µL of plasma for PK analysis. The total volume from each patient was limited to 3% of the total blood volume during a period of four weeks and did not exceed 1% at any single time (based on a total blood volume of 80 – 90 mL/kg body weight (3% = 2.4mL per kg each month) [80, 140]. Blood samples were collected from either a capillary heel prick or an arterial line. Central venous lines or long lines were not used

for samples due to the risk of contamination from a previous ciprofloxacin infusion. Additional samples were scavenged from blood collected for clinical care. The clinical teams were trained to record the exact time of the infusion and sampling to the minute.

Table 2-2 Timed PK blood sampling schedule

| **Sampling schedule group** | | **Sample time (T) after the infusion*** | | | | | **Blood volume mL Total** (over 7 days) |
|---|---|---|---|---|---|---|---|
| **weight >1000g** | **weight <1000g** | T1 - 3 mins after the end of the infusion<br>T2 – 12 = hours after the start of the infusion | | | | | |
| **A** | | T1 | T3 | | T8 | | 1.2 |
| | Ai | T1 | T3 | | | | 0.8 |
| | Aii | T1 | | | T8 | | 0.8 |
| | Aiii | | T3 | | T8 | | 0.8 |
| **B** | | | T2 | | T6 | T12 | 1.2 |
| | Bi | | T2 | | T6 | | 0.8 |
| | Bii | | T2 | | | T12 | 0.8 |
| | Biii | | | | T6 | T12 | 0.8 |
| **C** | | T1 | T3 | | T6 | | 1.2 |
| **D** | D | | T2 | T4 | T8 | | 1.2 |

2.2.1.5 Dose regimens

A commercial, generic preparation of ciprofloxacin solution 2 mg/mL either as 50 mL, 100 mL or 200mL bottles produced by Claris and supplied by Peckforton Pharmaceuticals (Cheshire UK) was dispensed as standard hospital stock. The NHS hospital pharmacy dispensed the IMP as standard stock therefore the MHRA confirmed there were no further requirements for drug accountability additional to NHS standards

Intravenous ciprofloxacin was administered as part of standard care in accordance with the local protocol for infection, usually as a second line rescue therapy for suspected Gram-negative infection. It was also given as the renal sparing drug of choice when there were early signs of renal failure due to nephrotoxicity associated

with the first line antibiotic gentamicin. The dose regimens was based on British National Formulary for Children prescribing guideline [173]:

Neonate <1 month 10mg/kg 12 hourly
Neonate >1 month 10mg/kg either 8 or 12 hourly
Renal compromise (creatinine >100 µmol/L) 5mg/kg 12 hourly

The decision to administer a higher or lower dose was at clinician's discretion. A lower dose of 5mg/kg/12 hourly was prescribed when there were signs of renal failure indicated by a creatinine >100 µmol/L. A higher dose 10mg/kg 8 hourly was prescribed for neonates >36 weeks PMA if indicated by the severity of illness.

To determine whether this dose needed adjustment an interim analysis was performed for the first ten babies. An increased dose was envisaged if the AUC/MIC ratio was below the expected target of 125. If more than 25% of patients exhibited an AUC/MIC <80, the dose would have been increased by 20% or if the AUC/MIC<50 the dose would have been doubled. A dose decrease would only have been supported if acute side effects were reported as monitored by the safety data monitoring board. PK analysis was carried out using the nonlinear mixed effects modelling program NONMEM VI (V2.0; GloboMax LLC, USA).

#### 2.2.1.6 Drug Administration

The intravenous infusion was prepared and administered according to routine clinical practice by central or peripheral intravenous line. The total drug volume and flush were administered over 30 minutes in the neonatal unit or 60 minutes in the paediatric intensive care unit. The exact time the infusion commenced and completed was recorded.

Standard operating procedures were developed specific to the drug administration method at each site. In the paediatric hospital the infusion line may be primed with either the drug or saline. When saline was used the time between the drug reaching the vein depended on whether the rate was set to include the drug and flush volume

(total mL/hour) or the rate (mL/hour) and drug volume. Adjustments were made according to the following formula to estimate the time the drug takes to reach the vein:

**a) Infusion line primed with ciprofloxacin**

The drug was drawn into the syringe then purged to prime the infusion line and the actual volume to be administered remained in the syringe. The line was then connected to the cannula**.** The pump was set to deliver the required volume of drug in the syringe (not the drug in the line). On completion the line primed with ciprofloxacin was disconnected from the cannula. A saline flush was connected directly into the cannula.

**b)** Infusion line primed with a saline (flush included in the total volume infused)

**Dead space volume (mL) =**

**infusion line** **+ the filter** **+ cannula**

Figure 2-1 Dead space between the infusion pump and the vein

The syringe with the exact volume of drug was connected to the line primed with saline. The rate of infusion was set to **include** the volume of drug to be administered and the flush volume (estimated as 2mL). A syringe with a 2mL saline flush was connected as soon as the pump detected the volume had been infused. The flush was then infused at the same rate to ensure the remaining drug in the dead space of the infusion line was delivered within the same period (30 or 60 minutes). The delay in the drug reaching the vein was calculated as in Table 2-3.

**c)** Infusion line primed with a saline (flush excluded from the total volume infused)

The syringe with the exact volume of drug to be administered was connected to a line primed with saline. The rate of infusion was set to exclude the flush volume (estimated as 2mL). A syringe with a 2mL saline flush was connected at the end of the infusion and administered at the same rate therefore the drug did not reach the vein until the initial flush has been infused. This delay was calculated as Table 2-4:

Infusion time (mins) / rate set on infusion pump x 2 (flush volume) =

= time before the drug reaches the vein.

Table 2-3 **Time for the drug to reach the vein (includes the flush volume)**

The line is primed with saline then infused over 60 minutes and includes the flush volume of 2mL

| Neonate's weight (kg) | Drug volume (mL) | Rate (mL/hour) | Delay before the infusion reaches the vein (mins) | Total time to administer (mins) |
|---|---|---|---|---|
| 1 | 5 | 7 | 17 | 77 |
| 2 | 10 | 12 | 10 | 70 |
| 3 | 15 | 17 | 7 | 67 |
| 4 | 20 | 22 | 5.4 | 65 |
| 5 | 25 | 27 | 4.4 | 64 |

Infusion time (mins) / rate set on infusion pump x 2 (flush volume) =

= time before the drug reaches the vein.

Table 2-4 **Time for the drug to reach the vein (excludes the flush)**

The rate excludes the flush volume of 2mL and the line is primed with saline then infused over 60 minutes

| Neonate's weight (kg) | Drug volume (mL) | Rate (mL/hour) | Delay before the infusion reaches the vein (mins) | Total time to administer (mins) |
|---|---|---|---|---|
| 1 | 5 | 5 | 24 | 84 |
| 2 | 10 | 10 | 12 | 72 |
| 3 | 15 | 15 | 8 | 68 |
| 4 | 20 | 20 | 6 | 66 |
| 5 | 25 | 25 | 4.8 | 65 |

#### 2.2.1.7 Laboratory analysis

Serum creatinine concentrations were measured at Alder Hey Children's NHS FT with an adapted Jaffé method and Architect C system (Abbott diagnostics, Illinois, US). The PK blood sample was allowed to clot for a period of 30 minutes or more then centrifuged at 4,000 rpm for 10 minutes at room temperature or 4°C within 24 hours of collection. The serum was transferred to a clean polypropylene tube/plain tube and stored at −20°C until shipped in batches to the PK laboratory.

A method suitable for assaying small plasma volumes was developed and implemented by the pharmacology laboratory University Hospital of Tours, France [243]. Ciprofloxacin concentrations were determined using high-performance liquid chromatography (HPLC) and quantification with mass spectrometry (LC-MS). D4–ciprofloxacin was the internal standard. The calibration curve ranged from 25 to 3000 ng/mL. The inter- and intra-day coefficients of variation (CVs) of controls were 4.1% and 2.4%, respectively. The lower limit of quantification was 25ng/mL.

#### 2.2.1.8 Screening and Recruitment

Daily screening of all admissions in the neonatal and paediatric intensive care units identified neonates who met the eligibility criteria. A log was kept detailing which parents/babies were approached, recruited or declined and the reason. As this was a population PK clinical trial the population included those treated with ciprofloxacin for suspected sepsis as part of their standard care rather than administering the drug for the purpose of research. Parental consent was requested primarily to collect PK blood samples and optional requests for sub-studies:

i) Collection of DNA samples

ii) CSF sample when a lumbar puncture was required for clinical care.

Consent for those prescribed ciprofloxacin and prospective consent for those identified as more likely to be prescribed ciprofloxacin at a later date. Administration of ciprofloxacin was not delayed by the consent process. To allow the first PK sample

to be taken within three minutes of the end of the first infusion of ciprofloxacin, the following approaches to consent were approved by National Research Ethics Service and European Ethics TINN:

**Prospective consent** - those more likely to commence ciprofloxacin were identified and routinely consented using the following criteria:

a) Screened for sepsis due to presenting clinical signs.

b) Extreme prematurity - born <28 weeks post menstrual age.

c) First line antibiotics for suspected Gram-negative infection.

**Telephone Consent** – when the information sheet had been provided to families, the investigator telephoned the family to request consent within a set process approved by NRES and witnessed by a member of the clinical team.

**Deferred Emergency Consent** - Amendment 2008 for Children in Emergency - UK SI 941 [240, 244] allowed the initial blood sample to be collected then request consent to use the data retrospectively. Data were only included in the analysis if the parent(s) subsequently gave informed consent. Every effort was made to request parental consent first. Last day samples, DNA samples, or CSF samples were not collected unless consent was subsequently obtained.

These consent processes are consistent with the Medicines for Human Use Clinical Trial Regulations (2004) and International Conference on Harmonisation Good Clinical Practice (ICH GCP)(1996) [239, 245] and UK law. Consent was obtained from a person with parental responsibility defined by the Children's Act 1989 and the Children and Adoption Act 2002 [246, 247]. The parent information sheet was designed with input from parents and approved by REC and included the twenty elements of consent [232]. Parent(s) were approached initially by the clinical team. Parents were given repeated opportunities to ask questions, clarify any issues about the study and confirm or withdraw consent. The informed consent process was delivered by health care professionals experienced in PICU/NICU, trained in ICH

GCP, consent law and the trial procedures then delegated by the Principal Investigator. A medically qualified doctor assessed the eligibility prior to any study procedure (required by the regulatory body MHRA).

#### 2.2.1.9 Cerebro-spinal fluid (CSF) sampling

To determine the CSF penetration of ciprofloxacin samples were collected if/ when a lumbar puncture was performed for clinical care following the administration of ciprofloxacin. Between 150 µL and 0.5 mL of CSF was collected in a sterile polypropylene 'plain' tube. Labels and CSF sample bottles were prepared and added to cots to ensure the clinical team were reminded at the time of sampling. The exact time of sampling was recorded by the clinical team and the CSF was stored immediately at -20°C.

### 2.2.2 Population PK modelling of ciprofloxacin

PK analysis was carried out by the Department of Clinical Pharmacology Robert Debre Hospital using the nonlinear mixed effects modelling program NONMEM V 7.2 (Icon Development Solutions, USA). First order conditional estimation (FOCE) method with interaction was used to estimate PK parameters and their variability.

Inter-individual variability of the PK parameters was estimated using an exponential model and was expressed as follows:

$\Theta_i = \theta_{mean} * e^{\eta i}$ where $\theta_i$ represents the parameter value of the $i^{th}$ subject, $\theta_{mean}$ the typical value of the parameter in the population and ηi the variability between subjects which is assumed to follow a normal distribution with a mean of zero and variance ω2.

The model was parameterised in terms of:

- V1 central volume of distribution
- V2 peripheral volume of distribution
- Q inter-compartmental clearance
- CL clearance

Inter–individual variability was described by an exponential model and was estimated for V1, V2 and CL. Inter occasion variability on CL was coupled to inter-individual

variability by an additive model. A proportional model best described residual variability.

#### 2.2.2.1 Covariate analysis

Covariate analysis followed a forward and backward selection process. The likelihood ratio test was used to test the effect of each variable on model parameters including:

current weight
birth weight
gestational age
postnatal age (when administered ciprofloxacin)
postmenstrual age (at birth)
serum creatinine concentration (within ≤48 hours of PK sampling)

Pre-specified co-medication included: inotropes, ibuprofen, diuretics, caffeine, teicoplanin, amoxicillin-clavulanic acid, nystatin and selective decontamination of the digestive tract (coliston, amphotericin, tobramycin)

During the first step of covariate model building, a covariate was included if there was a significant ($p<0.05$, $\chi 2$ distribution with one degree of freedom). This was indicated by a reduction of >3.84 in the objective function value (OFV) from the basic model. All the significant covariates were then added simultaneously to construct a 'full' model. Subsequently, each covariate was independently removed from the full model. If the increase in the OFV was >6.635 ($p<0.01$, $\chi 2$ distribution), the covariate was considered to be statistically significant therefore retained in the final model.

An allometric scaling function was used *a priori* to describe the effect of weight on clearance and volume average (allometric coefficients of 0.75 for CL and Q, for V1 and V2) (0.75 based on the average adult weight).

Model validation was based on graphical and statistical criteria. Goodness-of-fit plots, including observed (DV) *versus* population prediction (PRED); DV *versus* individual prediction (IPRED); conditional weighted residuals (CWRES) *versus* time and CWRES versus PRED were initially used for diagnostic purposes [248]. The stability

and performance of the final model was also assessed by means of a nonparametric bootstrap with re-sampling and replacement. Re-sampling was repeated 500 times and the values of estimated parameters from the bootstrap procedure were compared with those estimated from the original data set. The entire procedure was performed in an automated fashion, using PsN software (v2.30) [249].

#### 2.2.2.2 Model evaluation:

Model validation was based normalized prediction distribution errors (NPDE) [250]. Goodness-of-fit plots, including observed (DV) *versus* population prediction (PRED); DV *versus* individual prediction (IPRED); conditional weighted residuals (CWRES) *versus* time and CWRES *versus* PRED were initially used for diagnostic purposes. The stability and performance of the final model was also assessed by means of a nonparametric bootstrap with re-sampling and replacement. Re-sampling was repeated 500 times and the values of estimated parameters from the bootstrap procedure were compared with those estimated from the original data set. The entire procedure was performed in an automated fashion, using PsN (v2.30). One thousand datasets were simulated using the final population model parameters. NPDE results were summarised graphically by default as provided by the NPDE R package (v1.2) [251]: (i) QQ-plot of the NPDE; (ii) histogram of the NPDE. The NPDE is expected to follow the N (0, 1) distribution to show there is no bias in the model.

#### 2.2.2.3 Ciprofloxacin penetration into the cerebrospinal fluid

Assessment of the ciprofloxacin penetration into the cerebrospinal fluid (CSF) was evaluated by the $CSF/_{serum}$ ciprofloxacin concentration ratio. Because serum ciprofloxacin concentrations were not obtained concurrently with CSF sample collection, serum concentrations at the time of CSF sample collection were calculated from the Bayesian estimates for the concentration obtained from each individual patient.

#### 2.2.2.4 Simulation of Dose Regimens

Monte Carlo simulations were performed using the parameter estimates obtained from the final model in order to define optimal dosing regimens able to attain the target AUC/MIC of 125 in about 80% of patients. To ensure comparable safety profiles, the percentage of patients below the reported maximum AUC was also considered. The paediatric dose of ciprofloxacin was simulated on a mg/kg basis according to different age groups. Thus, various mg/kg dosing regimens (5, 7.5, 10, 12.5, 15 mg/kg twice daily) were simulated in each neonatal group. One thousand simulations were performed and $AUC_{0\text{-}24}$ at steady state was calculated for each simulated patient. The target attainment rate was then calculated for each dosing regimens to define the optimal dose regimens in each neonatal group. Two graphs illustrated the simulated dose regime for neonates above and below PMA 34 weeks then PMA 36 weeks as estimates of the changes due to nephrogenesis.

### 2.2.3 Pharmacovigilance

A risk assessment of the investigational medicinal product included a summary of the adverse reactions reported in the SmPC and MHRA Public Assessment Report, back ground papers, British National Formulary for Children [53, 173] inclusive of the wider group of fluoroquinolones Table 2-5.

Clinical data were collected regarding adverse events to evaluate the study outcome and to comply with pharmacovigilance reporting of Clinical Trial Investigating a Medicinal Product (CTIMP) [245] . The non-serious events in the protocol that required reporting were approved by the Sponsor. Pharmacovigilance reporting continued until week six following administration of ciprofloxacin. All events were followed up until satisfactory clinical resolution. Each patient was reviewed daily until three days following the last dose. This included reviewing a patient data system that collects data from routine blood values, vital signs, microbiology results and possible adverse events during this period. Confounding variables associated with treatment,

other medications and known adverse events were systematically collected in the case report form based on a risk assessment of the IMP. As most patients were critically ill they were intensively monitored with continuous monitoring of vital signs as part of standard care. Biochemistry/haematology and frequent blood gas analyses were carried out for routine care and assessed against validated laboratory normal values with specific monitoring of renal and hepatic parameters (set by the paediatric laboratory). The development of a framework for more proportionate pharmacovigilance reporting in critical illness was developed and is described in more detail in Chapter 7.

Table 2-5 Known Adverse Reactions for Ciprofloxacin Injection

| **Body System** | **Hazard** | **Likelihood** Rare (R) Low (L) Medium (M) High (H) |
|---|---|---|
| Joints | Arthropathy/ Tendinopathy | M |
| Vein | Phlebitis | L |
| Liver Function | Failure /pancreatitis | L |
| Renal Function | Failure<br>Crystalluria | L |
| Blood cells | Deranged | L |
| Gastrointestinal | Colitis if severe and persistent consider Clostridium Difficile | M |
| Anaphylaxis /Allergy | | R |
| Skin | Rash/photosensitivity | M |
| Cardiac | ventricular arrhythmia,<br>QT interval prolongation | R |
| Neurological | Convulsions | M |
| Syndromes | Stevens Johnson /Lyell | R |

#### 2.2.3.1 Serious Adverse Events

The Principal Investigators were required to report Serious Adverse Events (SAE) or Suspected Unexpected Serious Adverse Reactions (SUSARs) to the Sponsor within 24 hours. These are defined by the Medicines for Human Use Clinical Trial Regulations (2004) 1031[240] as:

- resulting in death
- life-threatening

- requiring hospitalisation or prolongation of existing hospitalisation
- resulting in persistent or significant disability or incapacity.

SAEs anticipated due to critical illness (including death) were exempt from the time frame associated with expedited reporting to the MHRA and NRES. Anticipated SAE were listed in the protocol based on the incidence at Liverpool Women's NHS FT between 1980 and 2004 Table 2-6. The Sponsor (Trust) monitored the incidence of anticipated SAE, an increase would require expedited reporting as a Suspected Unexpected Serious Adverse Reaction (SUSAR) due to a novel causal relationship not stated in the Summary of Product Characteristics [53]. The Principal Investigator was required to report all other SAE and SUSARs to the Sponsor within 24 hours. The Sponsor then determined whether the event required expedited reporting to the MHRA and REC within 7 or 15 days in accordance with regulation 33 [245] . Annual safety reports were submitted to the MHRA and NRES and SAE were reviewed by the Data Monitoring Committee (DMC) at regular intervals throughout the trial.

Table 2-6 Anticipated Serious Adverse Events for Neonates

| **Adverse event** | **Incidence** Weeks PMA: | |
|---|---|---|
| | **< 28** | **>28 -34** |
| Death | 20 | 8 |
| Necrotising enterocolitis or intestinal perforation | 15 | 3 |
| Intracranial abnormality | 15 | 6 |
| Patent ductus arteriosus | 25 | 8 |
| Retinal surgery for retinopathy of prematurity | 5 | 0.14 |
| Pulmonary haemorrhage | 5 | 0.5 |

#### 2.2.3.2 Arthralgia/Arthropathy

Parents and the clinical team who frequently handle the babies were asked to report any signs of joint inflammation or pain during handling until discharge or up to a period of six weeks. These terms describe musculoskeletal adverse events including the commonest symptom arthralgia 'pain' (50%), tendon or joints disorders (19%) and reduced movement (15%) [190]. This pragmatic approach reflects clinical practice as it is vital to minimise disturbing neonates and there is no validated method for assessing arthralgia in non-weight bearing neonates.

#### 2.2.3.3 Tolerability - Peripheral Infusion Site

Tolerance at the site of peripheral infusion was monitored immediately before and after the infusion using the validated Visual Infusion Phlebitis Scale [207, 208] cited by Gallant and Schultz [209] **Table 2-7**. Phlebitis was assessed by monitoring pain, erythema (redness or rash), induration (hardened skin), swelling and palpable venous cord on a scale of 0-5 at three time points immediately before and on completion of the 30 - 60 minute infusion then six hours later. This group were administered other drugs and fluids through the same intravenous site.

**Table 2-7 Phlebitis** Scale 1- 5

| Assessment | Score | Action |
|---|---|---|
| IV Site appears healthy | 0 | No sign of phlebitis observe cannula |
| Slight pain or redness near IV site | 1 | Possibly first sign of phlebitis observe cannula |
| **2 of the following:**<br>Pain at IV site when touched<br>Erythema<br>Swelling | 2 | Early stage of phlebitis<br>Re-site cannula |
| **All 3 signs are evident & extensive**<br>Pain along path of cannula<br>Erythema<br>Induration | 3 | Medium stage of phlebitis re-site cannula and consider treatment |
| **All 4 signs are evident & extensive**<br>Pain along path of cannula<br>Erythema<br>Induration<br>Palpable venous cord | 4 | Advanced stage of phlebitis or start of thrombophlebitis<br>Re-site cannula<br>Consider treatment |
| **All 5 signs are evident & extensive**<br>Pain along path of cannula<br>Erythema<br>Induration<br>Palpable venous cord<br>Pyrexia | 5 | Advanced stage of Thrombophlebitis<br>Initiate treatment<br>Re-site cannula |

### 2.2.4 Research Culture

This was the first population PK clinical trial at either of the recruiting sites. To ensure parents were not overburdened by research a co-ordinated approach to participation in research was developed within the neonatal unit. Parents were informed of all studies that they may be approached for at the outset and a research poster board included a lay summary of each trial in the parent's rest room. Over this period four other clinical trials were conducted involving this population.

To establish a PK culture the clinical teams including over 200 staff in each intensive care unit were trained to adhere to the precise sampling and drug administration methods and provide support to families. This included pharmacists, phlebotomists, nurses and medical staff providing care 24/7 care. Laboratory technicians were GCP/GLP trained and trained in study specific standard operating procedures by the research fellow. At the 40 non-recruiting sites the PI's and research nurses were GCP trained and provided with simplified SOPs specific to the activities at the follow up sites. Further guidance was provided at the time of transfer.

### 2.2.5 Regulations and Governance

A Clinical Trial Authorisation (CTA) was approved in accordance with the Medicines for Human Use Clinical Trial Regulations (2004) as the aim is to verify/compare the effect of the drug on the body including absorption, distribution, metabolism and excretion PK clinical trials are classed as CTIMP [240, 245]. The UK National Research Ethics Service approved the trial and subsequent amendments. The trial was registered with the European Clinical Trials Database Registry (EudraCT:2010-019955-23). The TINN Consortium European Ethics Advisory Board approved the study. A generic site specific information form was approved by the Comprehensive Research Network Service for Central NHS Permissions for forty continuing care sites with a statement of responsibilities specific to follow up. A Material Transfer Agreement covered the transfer of samples to Robert Debre Hospital, Paris for PK analysis and the DNA samples to University Ulm, Germany.

The storage and management of data was compliant with GCP, Data Protection Act and the National Information Governance Board [252]. The trial master file included version controlled essential documents [233]. Source data was obtained from BADGER and Meditech electronic patient data management systems and medical notes. Anonymised data was entered into a GCP compliant electronic case report form (Clininfo SA France). A unique study number was assigned to link to clinical

source documents with patient identifiable data. Medical notes and the tables linking unique study numbers to patients will be archived for 15 years using the NHS hospital appointed archiving services. GCP required that data was not extracted from Clin-info for reporting until data has been examined and locked by the Sponsor.

### 2.2.6 Oversight and monitoring

An independent Data Monitoring Committee (DMC) was established and the charter followed the DAMOCLES statement and safety reports sent every six months on safety [253]. The Sponsor (Trust R&D Department) monitored the study including compliance with GCP, Research Governance, source data checks, the laboratory handling of samples and data reliability. The case report forms were checked with multiple sources to verify sample times/infusions/or protocol deviations including the blood gas analyser (Rapid Lab Co-oximeter 1265 -Bayer UK), laboratory and clinical records. Serious breaches were minimised by preparing detailed standard operating procedures, trial tools and staff training provided by the research fellow. Breaches that affected the safety of the subject or the scientific integrity of the trial were required to be reported to the Sponsor (Trust R&D Policy) as per Regulation 29A [240]. A serious breach identified by the Sponsor (Trust) required reporting to the MHRA within seven days.

## 2.3 Pharmacokinetic Clinical Trial Results

Recruitment commenced February 2011 and the target of 50 was exceeded. In total 63 neonates and young infants up to 48 weeks PMA were recruited by October 2012. Recruitment continued above the initial target as requested by the TINN Consortium partly to gain more last day samples. The target for neonates (n=30) at Liverpool Women's NHS FT was exceeded as 63% of those eligible were recruited mainly in the premature age group (n=37). The target at Royal Liverpool Children's NHS FT was 20, 43% of those eligible were recruited mainly term babies (n=25). Each of the seven PMA sub-age groups represented by seven to thirteen babies but excluded those 49-52 weeks PMA (as ciprofloxacin was not prescribed to this age group). One patient withdrew from the study and one was excluded as peritoneal dialysis commenced and one had previous administration of ciprofloxacin within 24 hours. Over 2539 babies were screened on each day of their admission. The mean (SD) for postmenstrual age (PMA) when commencing PK sampling was 35.7 (6.5) (range 24.9–47.9) weeks and weight 2060 (1020) (range 700–4200) grams (n=60). PMA and current weight were all normally distributed (p=0.4 and p=0.2, respectively).

### 2.3.1 Interim analysis

As part of an interim analysis the proposed neonatal regimen of 10mg/kg 12 hourly was assessed for the first ten neonates recruited from Liverpool Women's NHS FT. The aim was to evaluate whether the minimum $AUC_{24}$/MIC ratio 1:125 required to optimise the probability of target attainment for therapeutic success informed by adult PD data was achieved by this dose. The mean (SD) postmenstrual age (PMA) was 31.74 (4.7) weeks (range 24.86 – 38.9 weeks), postnatal age (PNA) 34 (28) (range 8 -91) and mean weight (SD) was 1398 (601) g (range 720 -2630 g) Table 2-8.

Ciprofloxacin serum and plasma concentrations from 44 PK samples and 35 scavenged samples were measured (n=79) Table 2-9 . Concentrations ranged from 830–9,630 ng/mL for PK samples and 58.4–8,850 ng/mL for scavenged samples.

Four babies did not have last day (day 5-7) samples due to death or ciprofloxacin being stopped earlier. The subject LW8 had a higher concentration at T3 than T1 following intravenous administration. The $AUC_{24h}$/MIC ratio was higher than the minimum recommended ratio 1:125 [134, 176], for a dosing regimens of 10mg/kg twice daily. Simulated $AUC_{24h}$ was calculated as ratio of daily dose/CL. AUIC was estimated as the ratio of simulated $AUC_{24h}$/MIC.

Based on these results the Trial Management Group and TINN Consortium decided to continue with the planned regimens for the main clinical trial of 10 mg/kg 8 or 12 hourly except when signs of renal impairment were present (creatinine >100 µmol/L) then 5 mg/kg 12 hourly was prescribed.

Table 2-8 Characteristics of neonates in the **Interim Analysis** (n=10)

| **ID** | **PMA** (weeks) | **PNA** (days) | **Weight** (g) | **Serum creatinine** (µmol/L) |
|---|---|---|---|---|
| 1 | 30 | 42 | 1040 | 151 |
| 2 | 36.4 | 73 | 1960 | 43 |
| 3 | 32.1 | 26 | 1390 | 39 |
| 4 | 28.6 | 15 | 1025 | 54 |
| 5 | 29.6 | 28 | 1150 | 35 |
| 6 | 30.7 | 22 | 1260 | 40 |
| 7 | 27.8 | 20 | 850 | 24 |
| 8 | 38.8 | 8 | 2630 | 41 |
| 9 | 38.4 | 91 | 1950 | 25 |
| 10 | 24.8 | 11 | 720 | 53 |
| Median | 30.36 | 24 | 1205 | 41 |
| Min | 24.86 | 8 | 720 | 24 |
| Max | 38.86 | 91 | 2630 | 151 |
| Mean | 31.74 | 34 | 1398 | 51 |
| SD | 4.70 | 28 | 601 | 37 |

Table 2-9 Individual Bayesian estimated PK parameters –**Interim Analysis**

| ID | CL (L/h) | V1 (L) | V2 (L) | Q (L/h) | Dose (mg) | Dose (mg/L) | $AUC_{24}$ (mg*h/L) | $AUC_{24}$/ MIC |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.146 | 0.608 | 3.235 | 5.733 | 8.4 | 8.1 | 114.9 | 229.8 |
| 2 | 0.292 | 2.387 | 2.997 | 0.821 | 18 | 9.2 | 123.4 | 246.8 |
| 3 | 0.182 | 1.647 | 1.173 | 0.509 | 14 | 10.1 | 153.5 | 307 |
| 4 | 0.105 | 1.012 | 0.765 | 0.337 | 9.5 | 9.3 | 181.3 | 362.6 |
| 5 | 0.203 | 1.676 | 1.126 | 0.049 | 11.5 | 10 | 113.3 | 226.6 |
| 6 | 0.260 | 1.581 | 1.133 | 0.359 | 12.6 | 10 | 97.0 | 194 |
| 7 | 0.160 | 0.705 | 1.029 | 0.442 | 8.15 | 9.6 | 101.9 | 203.8 |
| 8 | 0.401 | 5.779 | 0.451 | 0.240 | 23 | 8.7 | 114.8 | 229.6 |
| 9 | 0.504 | 4.569 | 3.886 | 0.267 | 18 | 9.2 | 71.4 | 142.8 |
| 10 | 0.120 | 0.599 | 0.680 | 0.424 | 5.6 | 7.8 | 93.5 | 187 |
| median | 0.193 | 1.614 | 1.129 | 0.391 | | | | |
| min | 0.105 | 0.599 | 0.451 | 0.049 | | | | |
| max | 0.504 | 5.779 | 3.886 | 5.733 | | | | |

**MIC based on EUCAST's clinical breakpoint 0.5mg/L*

Table 2-10 PK parameters, simulated regimens and infusion time **Interim Analysis**

| | **Percentiles** | **10 mg/kg** 12 hourly (30 min infusion) | **10 mg/kg** 12 hourly (60 min infusion) | **5 mg/kg** 12 hourly (30 min infusion) |
|---|---|---|---|---|
| $AUC_{24h}$ (mg*h/L) | 5% | 80 | 80 | 40 |
| | **Median** | **123** | **123** | **61** |
| | 95% | 189 | 189 | 94 |
| $C_{max}$ (ng/mL) | 5% | 3420 | 1710 | 1710 |
| | **Median** | **7077** | **3540** | **3540** |
| | 95% | 13552 | 6804 | 6804 |
| $C_{min}$ (ng/mL) | 5% | 751 | 773 | 375 |
| | **Median** | **1516** | **1547** | **758** |
| | 95% | 2499 | 2539 | 1250 |

### 2.3.2 Main Pharmacokinetic Clinical Trial Results

Sixty three babies were recruited, one was subsequently withdrawn by parents and two found to be ineligible. A further thirty were consented prospectively were not recruited either because they were not prescribed ciprofloxacin or their target age group was completed when they became eligible.

A high ratio between screened and eligible patients resulted from the systematic screening of all neonates <52 weeks of age (each day during their admission). The optimal time for recruitment was before the first dose therefore screening aimed to identify participants who were at greater risk of Gram-negative sepsis classed as those more likely to commence ciprofloxacin to allow prospective consent as follows:

- Screened for sepsis due to presenting clinical signs.
- Extreme prematurity - born <28 weeks post menstrual age.
- First line antibiotics for suspected Gram-negative infection.

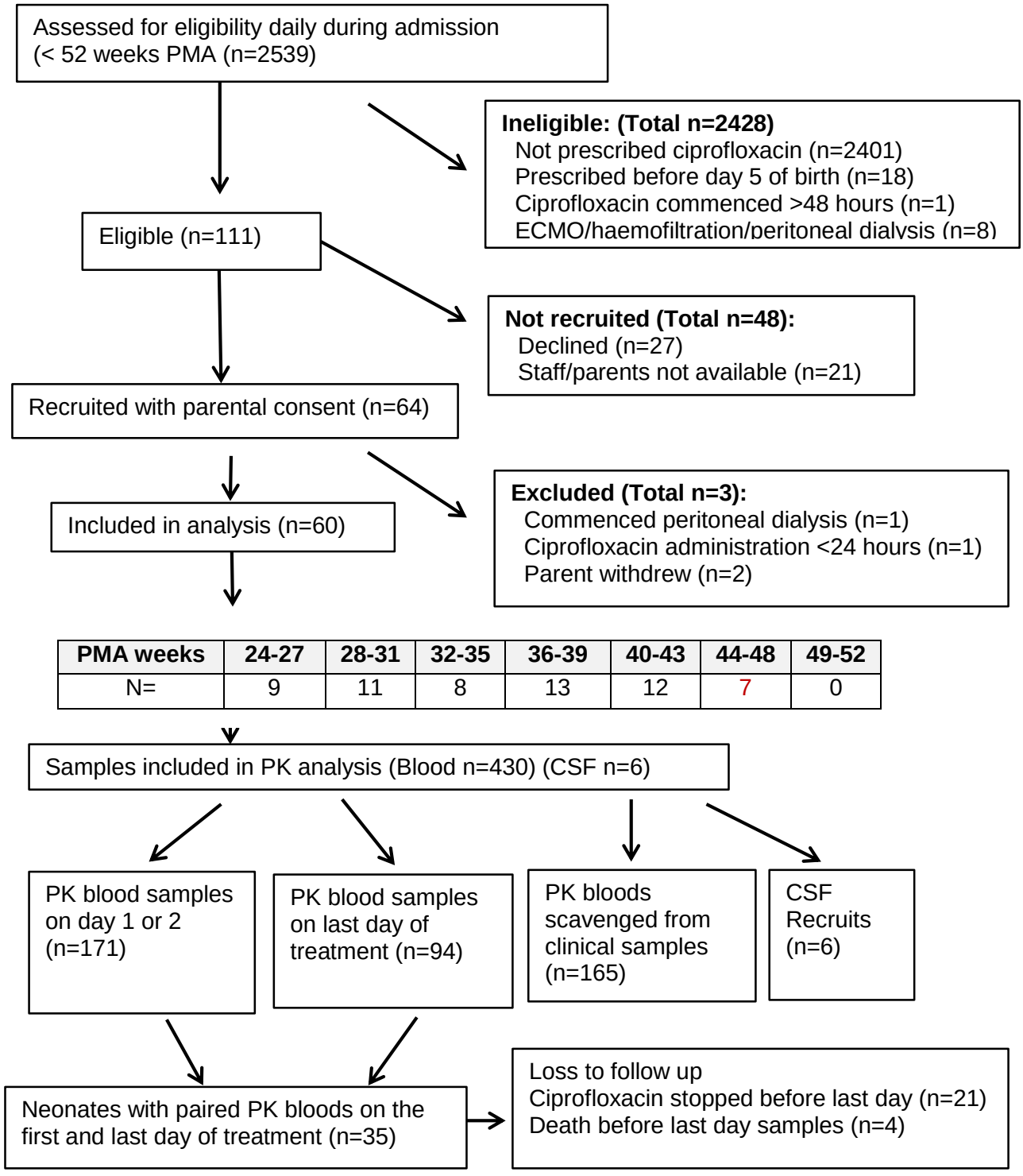



Figure 2-2 Consort flow diagram Pharmacokinetic Clinical Trial

This figure depicts the passage of all recruited and eligible participants through the clinical trial that contributed to a population PK analysis.

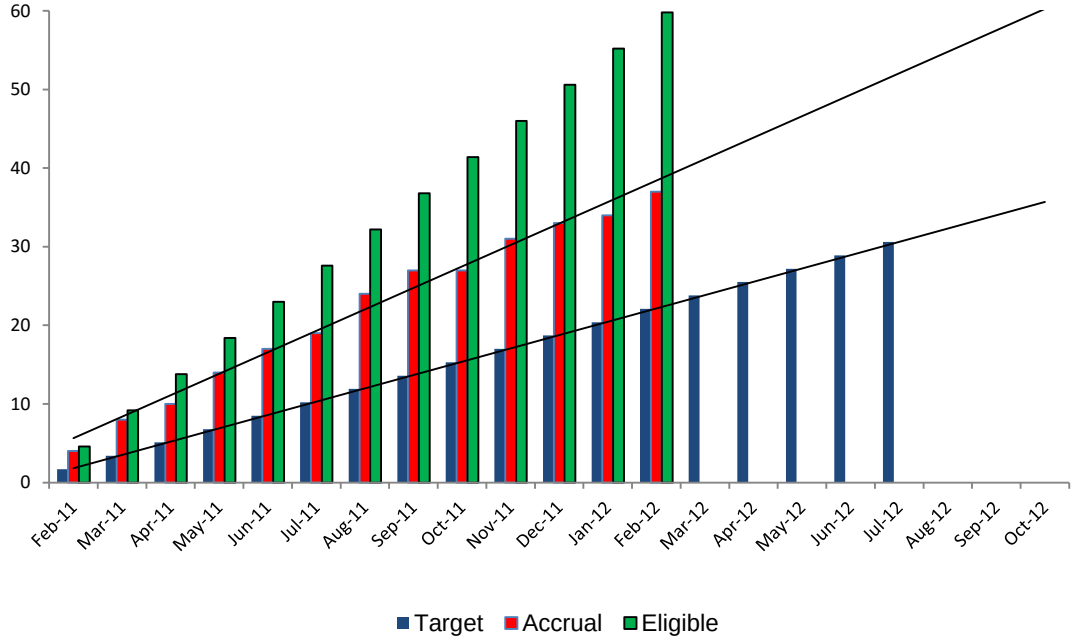



Figure 2-3 Neonatal Accrual Liverpool Women's NHS FT (n=37)

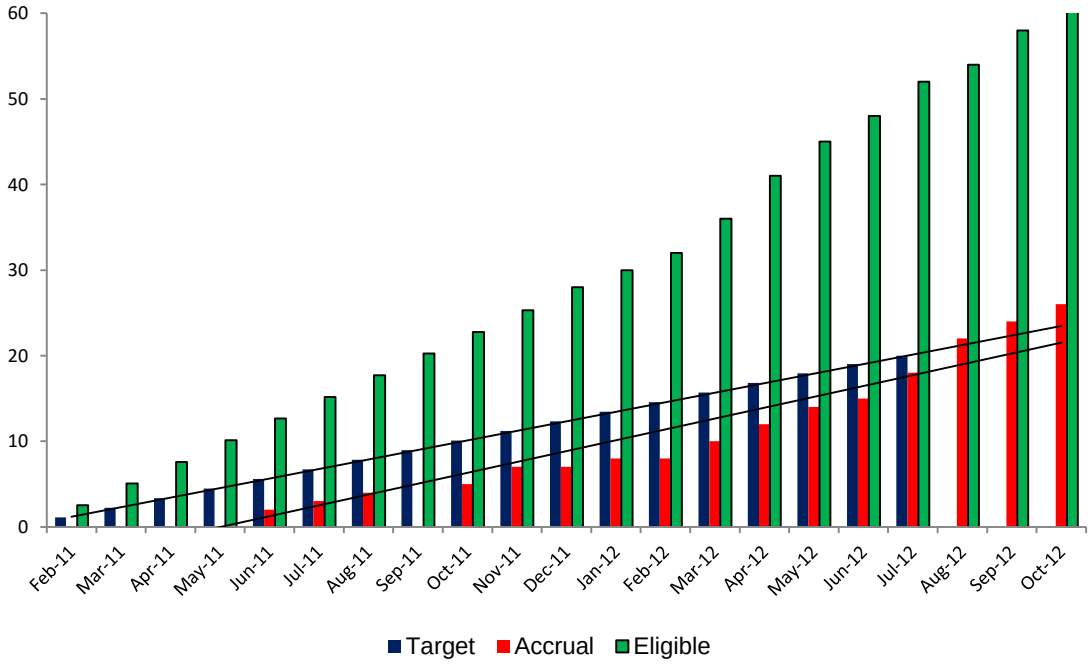



Figure 2-4 Paediatric Accrual Alder Hey Children's NHS FT (n=26)

Table 2-11 Demographic and Baseline Characteristics

| Recruits | N | (%) | Mean (SD) | | Median (Range) | |
|---|---|---|---|---|---|---|
| **Total recruited** | 64 | | | | | |
| withdrawn | 4 | | | | | |
| Proportion **o**f those eligible: | | | | | | |
| Neonatal hospital | | (63) | | | | |
| Paediatric hospital | | (43) | | | | |
| Gender: male | 39 | (62) | | | | |
| Race: | | | | | | |
| Asian | 5 | (8) | | | | |
| Caucasian | 53 | (88) | | | | |
| Unknown | 2 | | | | | |
| | | | | | | |
| **Age and weight:** | | | | | | |
| Post Menstrual Age: at birth (weeks) | | | 30.4 | (5.8) | 27.9 | (23.3-42) |
| Post-Natal Age: when recruited (days) | | | 38 | (30) | 27 | (5-121) |
| Post Menstrual Age: recruited (weeks) | | | 35.7 | (6.5) | 36.5 | (24.9-47.8) |
| IUGR | 3 | | | | | |
| Birth Weight (g) | | | 1518 | (884) | 1115 | (540-3850) |
| Weight (g) when recruited | | | 2060 | (1020) | 1955 | (700-4200) |
| Neonates weight <1000g | 9 | (14.5) | | | | |
| | | | | | | |
| **Mortality:** | | | | | | |
| Born < 28 weeks PMA | 7 | (11) | | | | |
| Born > 28 weeks PMA | 1 | (1.6) | | | | |
| | | | | | | |
| **Ciprofloxacin Administration:** | | | | | | |
| Duration (days) | | | 5 | (4) | 5 | (1-17) |
| Dose (mg/dose) | | | 18.9 | (10.1) | 18.7 | (4.5- 40.0) |
| Dose (mg/kg/dose) | | | 9.1 | (1.6) | 9.7 | (4.4-11.0) |
| Broviac line in situe | 16 | | | | | |
| | | | | | | |
| **Other:** | | | | | | |
| Admitted to Intensive care | 58 | (94) | | | | |
| Ventilated during PK sampling | 50 | (83) | | | | |
| Serum Creatinine (µmol/L) | | | 52 | (32) | 41 | (22 -164) |
| | | | | | | |
| **Pre-defined in the protocol co-medication*** | | | | | | |
| Inotropic agents | 22 | | | | | |
| Teicoplanin | 41 | | | | | |
| Diuretics | 30 | | | | | |
| Ibuprofen | 2 | | | | | |
| Caffeine | 15 | | | | | |
| Amoxicillin-Clavulanic acid | 12 | | | | | |
| Nystatin | 12 | | | | | |
| Colistin/Tobramycin/Amphotericin | 10 | | | | | |

* patients were administered other medication but these were specified in the protocol

### 2.3.2.1 Age and Weight

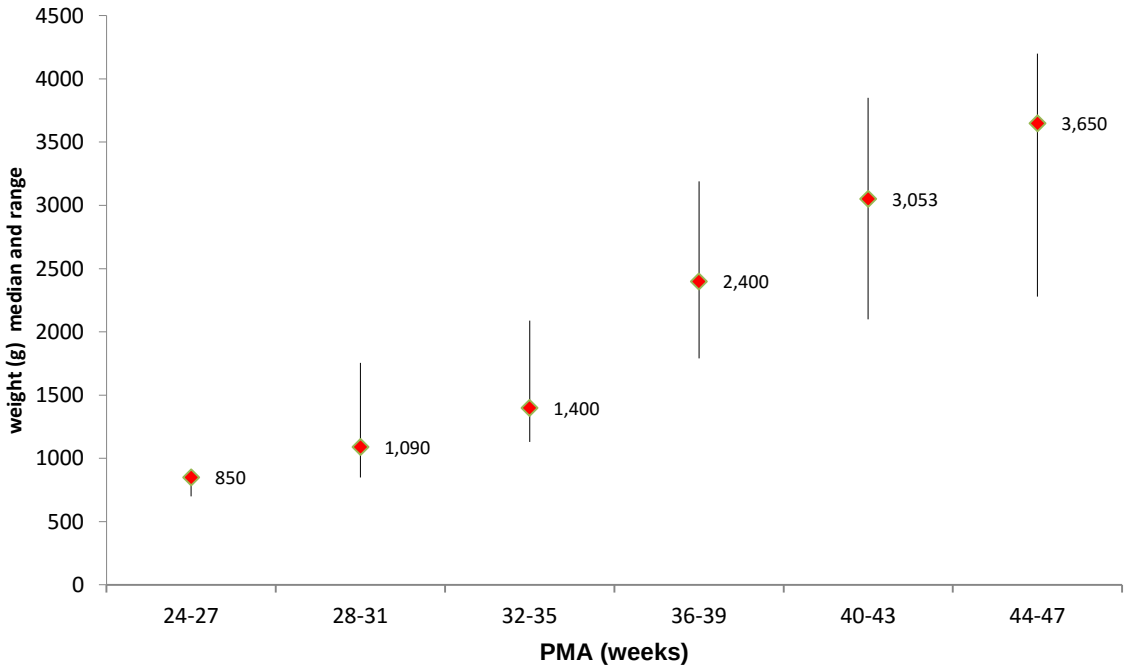
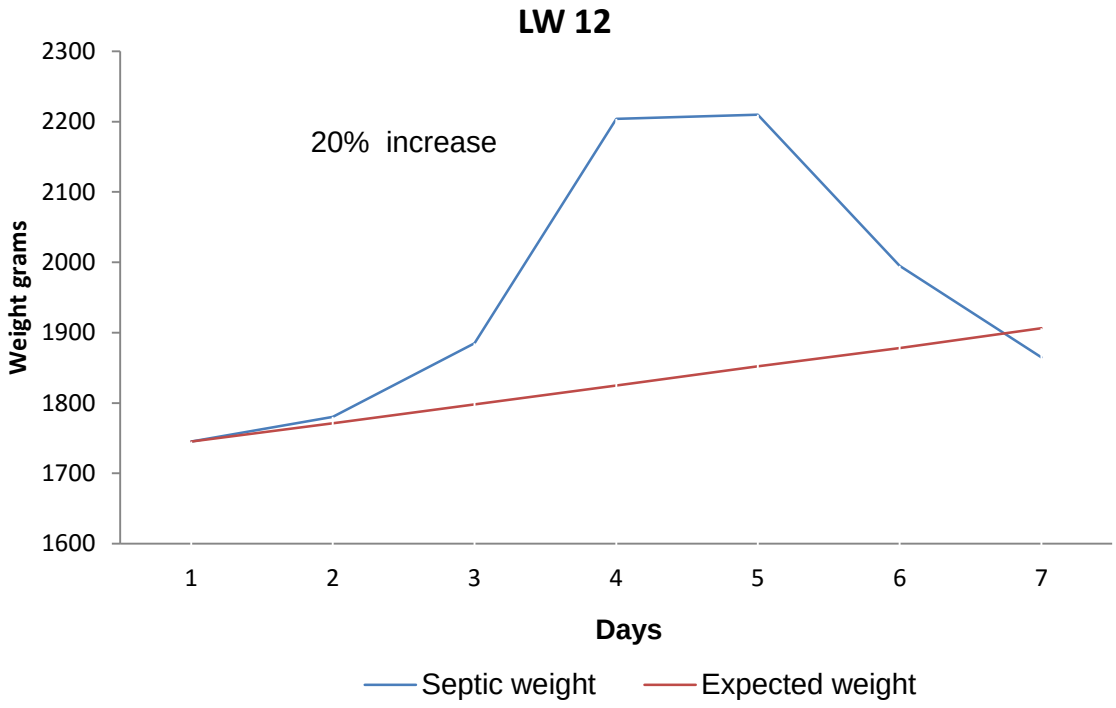



Figure 2-5 Weight (grams) range for each PMA Group

The weight range for the recruited babies was 700g – 4200g (median 1955g). The weight range at birth was 540 -3850g (median 1115g). The mean (SD) post menstrual age was 35.7 (6.5) range 24.9-47.9 weeks when recruited. PMA and current weight were distributed normally (p=0.4) and (p=0.2) respectively (Kolmogorov-Smirnov test). A weight increase >20% was evident for six neonates (20%) over the pre and post sepsis period. Figure 2-6 illustrates two examples of pre and post sepsis weight where weight increased by between 20% and 38% more than the neonates expected weight (expected weight gain of premature neonates is estimated as 15g/kg/day).



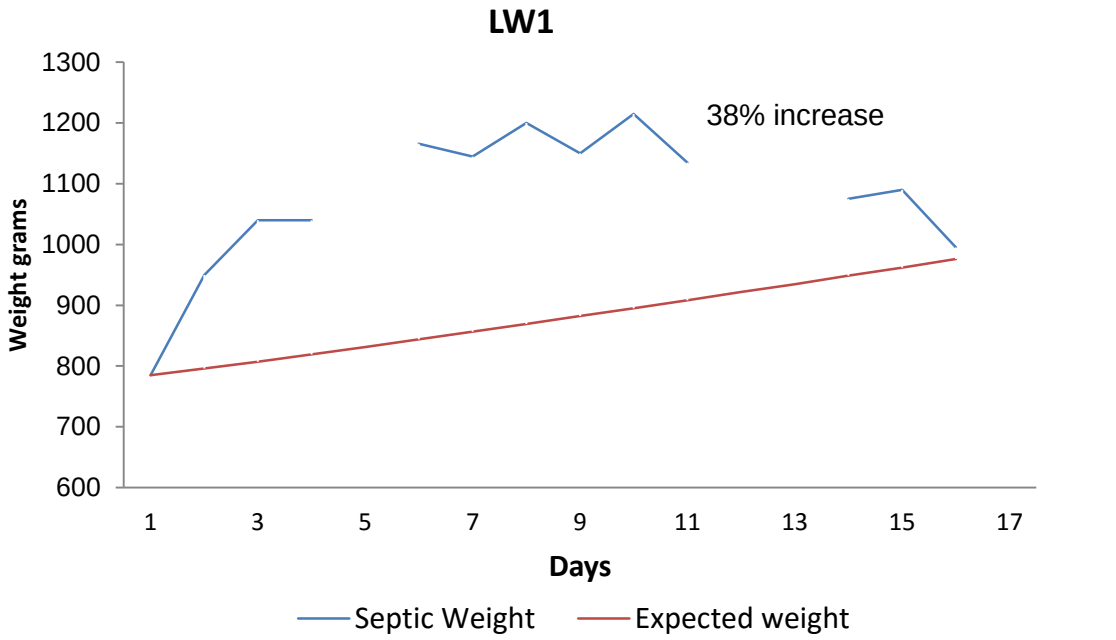




Figure 2-6 Variation in individual weight pre and post sepsis

### 2.3.2.2 Ciprofloxacin Administration

Ciprofloxacin was administered either over 30 minutes at Liverpool Women's (Neonatal) NHS FT or 60 minutes at Alder Hey (Paediatric) NHS FT. The regimens were determined by clinicians as per the sites policy, in most cases 20mg/kg/day (76%) Table 2-12. A renal sparing dose was prescribed 10mg/kg/day for six neonates and eight term babies received the higher regimen of 30mg/kg/day.

Table 2-12 Dose regimens administered mg/kg/day (n=60)

| PMA (weeks) | Dose Regimens (mg/kg/day) (%) | | |
|---|---|---|---|
| | 10 | 20 | 30 |
| **24-27** | 1 | 8 | |
| **28-31** | 2 | 9 | |
| **32-35** | 2 | 6 | |
| **36-39** | 1 | 12 | |
| **40-43** | | 9 | 2 |
| **44-48** | | 2 | 6 |
| **Total** | **6 (10)** | **46 (76)** | **8 (14)** |

### 2.3.2.3 Co-administration of Inotropes

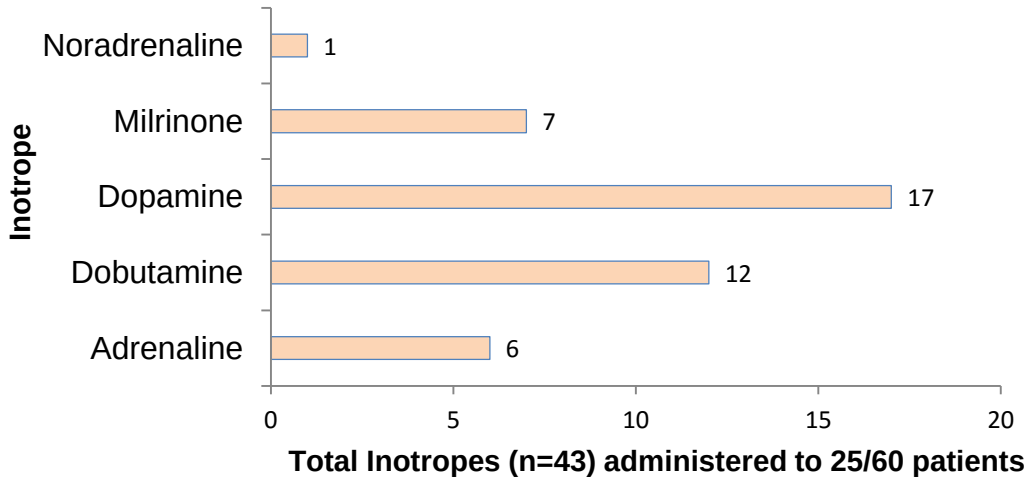



Figure 2-7 Type of Inotropes administered

Inotropes were administered to 22/60 (37%) neonates. Each neonate was administered up to three types of inotrope concomitantly. Post-operative cardiac patients were administered Milrinone.

2.3.2.4 Blood sample collection

A total of 60 neonates had PK samples collected at the pre-specified protocol times on the 1st or 2nd day and last day (n=268). Most samples (80% were collected on the first day either within three minutes or two hours of the first infusion depending on the group allocated. Fewer neonates 35 (58%) had samples on the last day of treatment. This loss was due to mortality or early discontinuation of treatment when the clinical signs of sepsis had resolved or when antimicrobial therapy was changed following laboratory confirmation of the organism. A further 168 samples were scavenged from clinical blood samples with precise infusion and collection times for up to a ten day period following the commencement of ciprofloxacin. In total 436 samples were collected, an average of 7.3 samples per neonate including 4.5 timed PK samples plus 2.8 scavenged from clinical bloods.

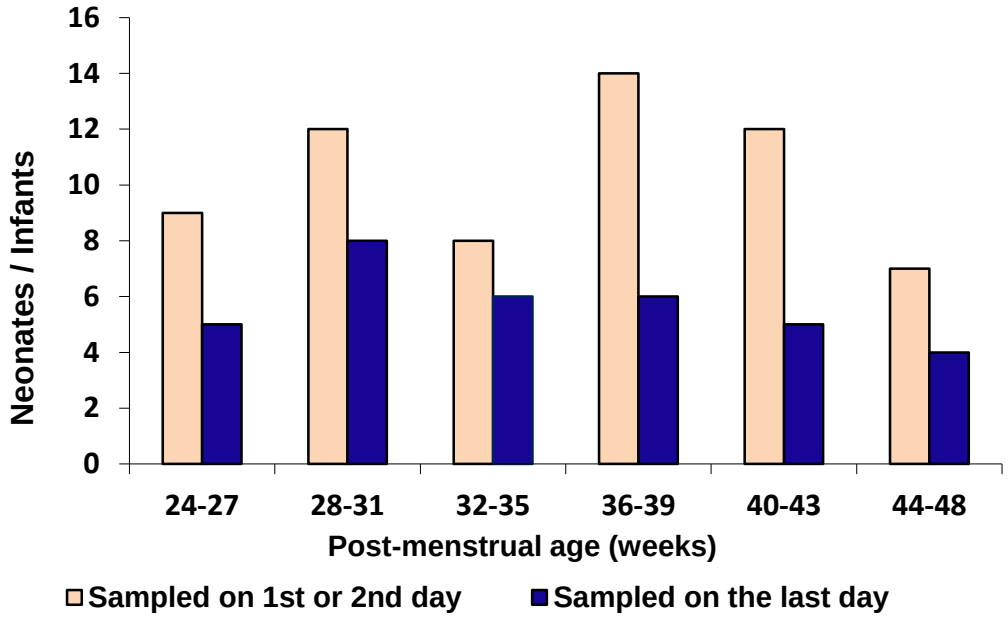



Figure 2-8 Neonates /Infants Sampled and Post Menstrual Age

Includes three patient who were subsequently withdrawn (n=63)

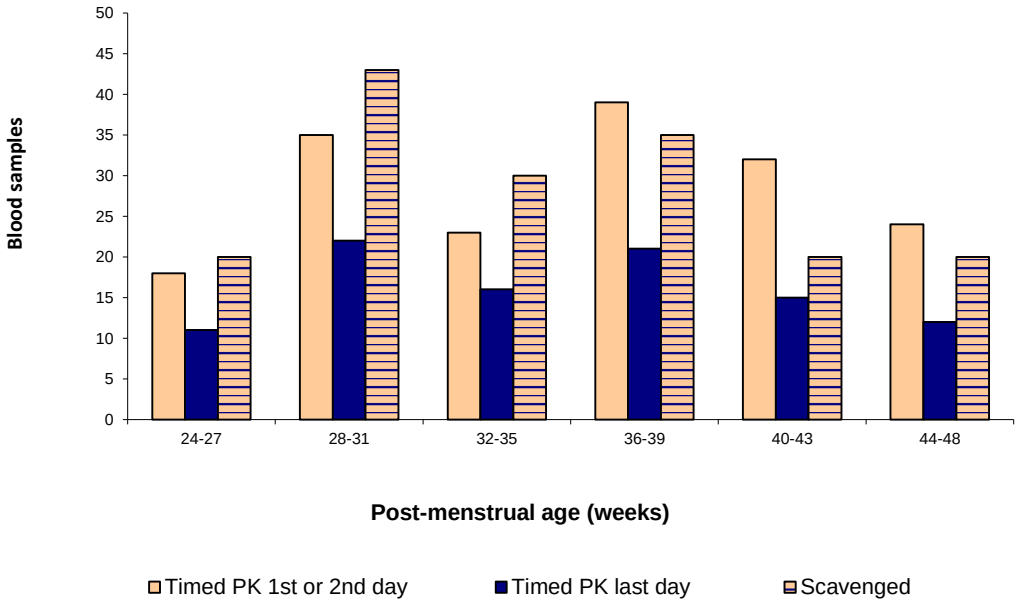



Figure 2-9 Blood samples representing each PMA Group

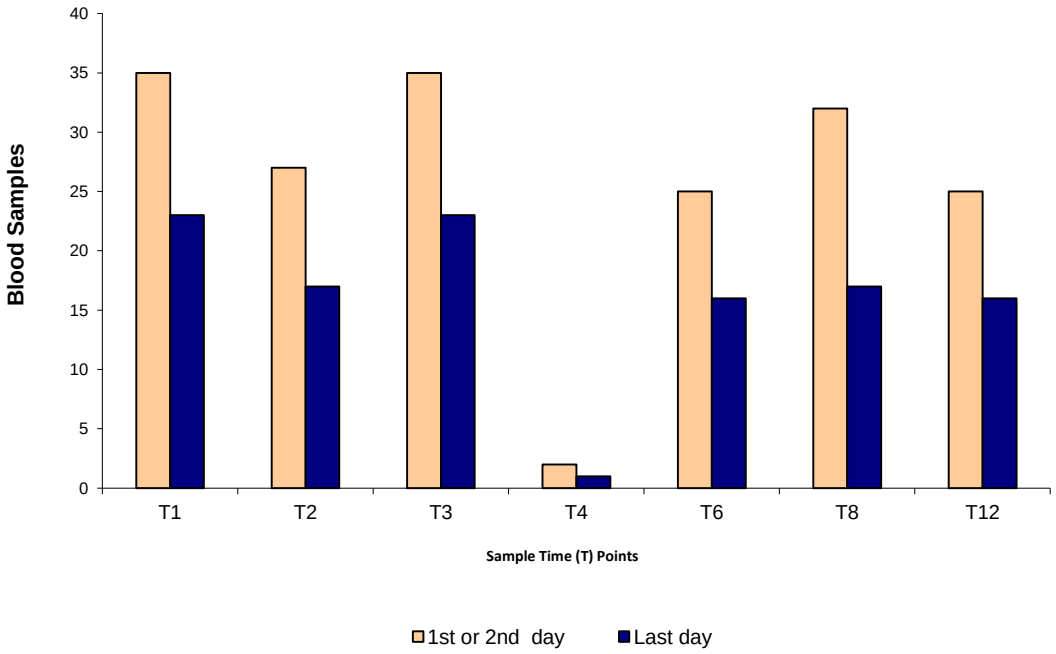



Figure 2-10 Distribution of population PK samples over time

The number of samples collected were distributed over informative time points on the 1st or 2nd day (n= 171) and on the last day (n=97). T1 was collected within 3 minutes of the first infusion and all other planned times were within 10 minutes of the protocol time. (T4 replaces T6 when prescribed 8 hourly rather than 12 hourly).

### 2.3.3 Pharmacokinetic Parameters

#### 2.3.3.1 Ciprofloxacin concentrations

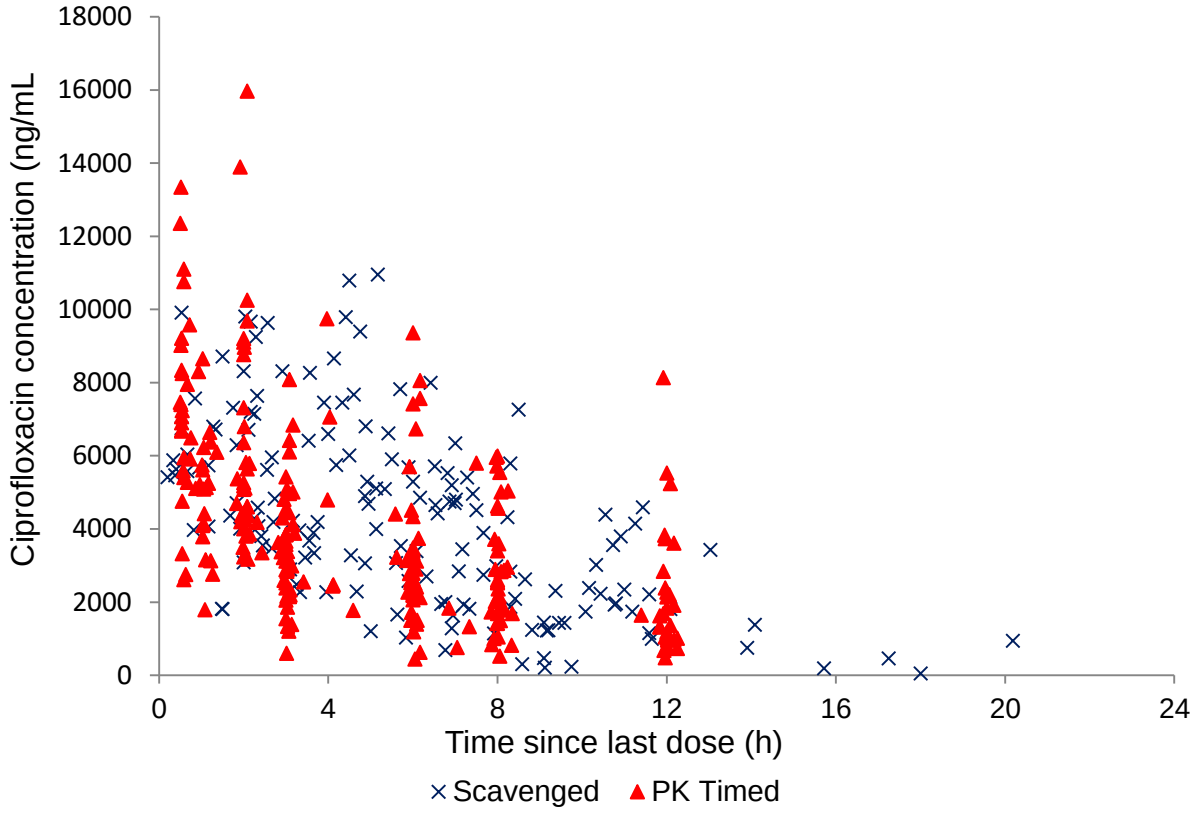



Figure 2-11 PK timed and Scavenged Samples concentrations

PK samples collected at set protocol time points (n=265) and scavenged samples (n= 165) *v* time. Total (n=430).

PK timed sample concentrations range 450–15976 ng/mL (median 3505 25$^{th}$ – 75$^{th}$ 2191 -5428). Mean 4120 (SD 2660)

Scavenged samples concentrations range 52–10961 ng/mL (median 4066; 25$^{th}$ – 75$^{th}$ 2212 – 5748). Mean 4257 (SD 2517)

(1 mg/L = 1000 ng/ml)

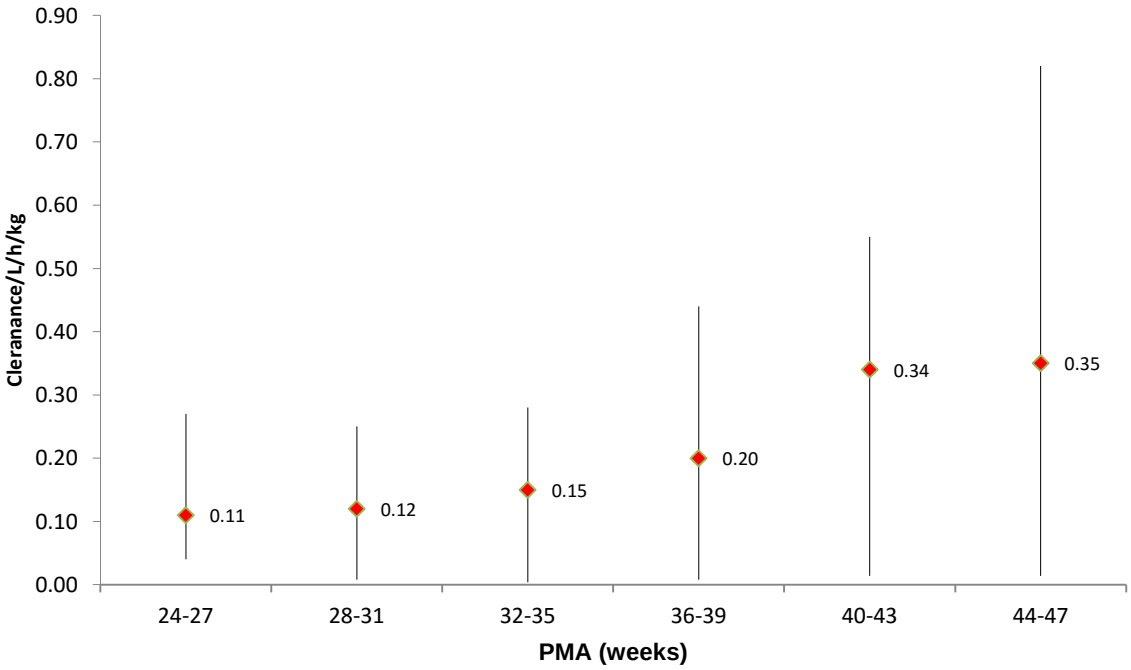



Figure 2-12 Clearance L/h/kg and PMA Group (weeks)

Clearance ranged from a minimum of 0.04 L/h/kg to max 0.81 L/h/kg
(each group's median and range is based on the median weight (n=60)

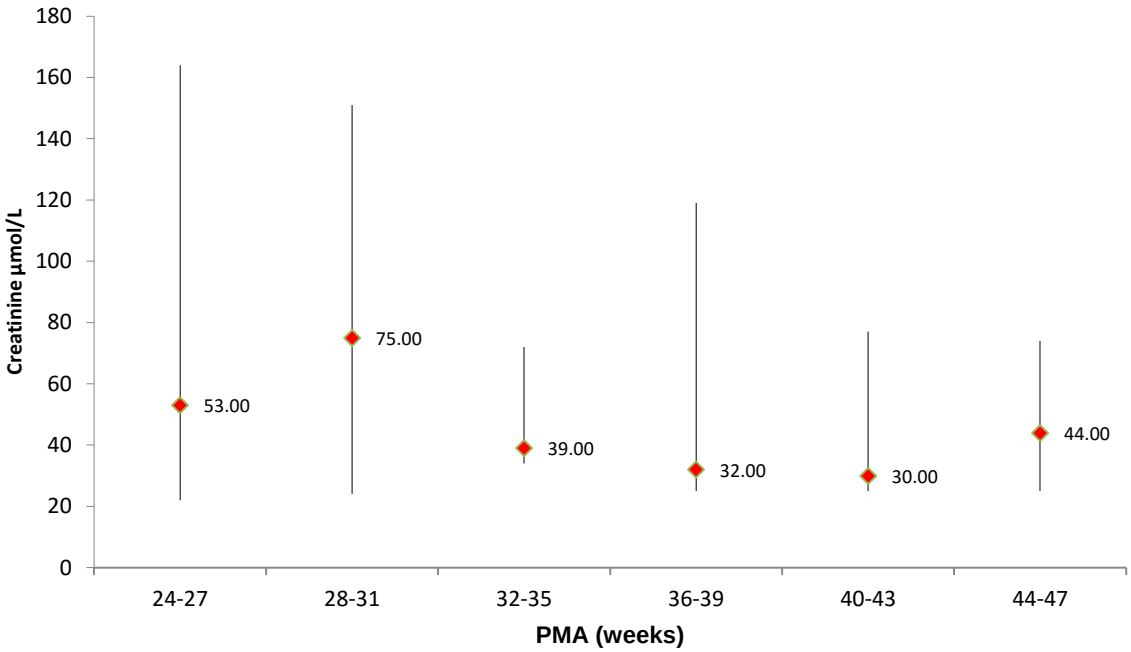



Figure 2-13 Creatinine µmol/L when commencing ciprofloxacin

Median and range for each PMA Groups when commencing ciprofloxacin (n=60)

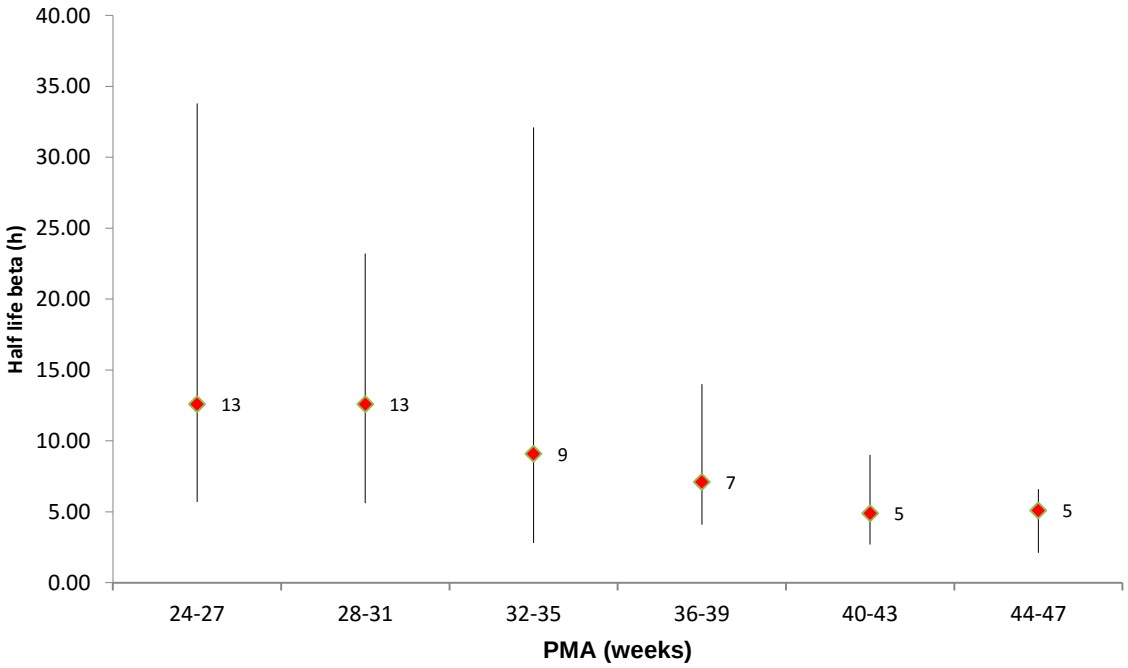



Figure 2-14 Half- Life Beta (h) and PMA Group
(N=60)

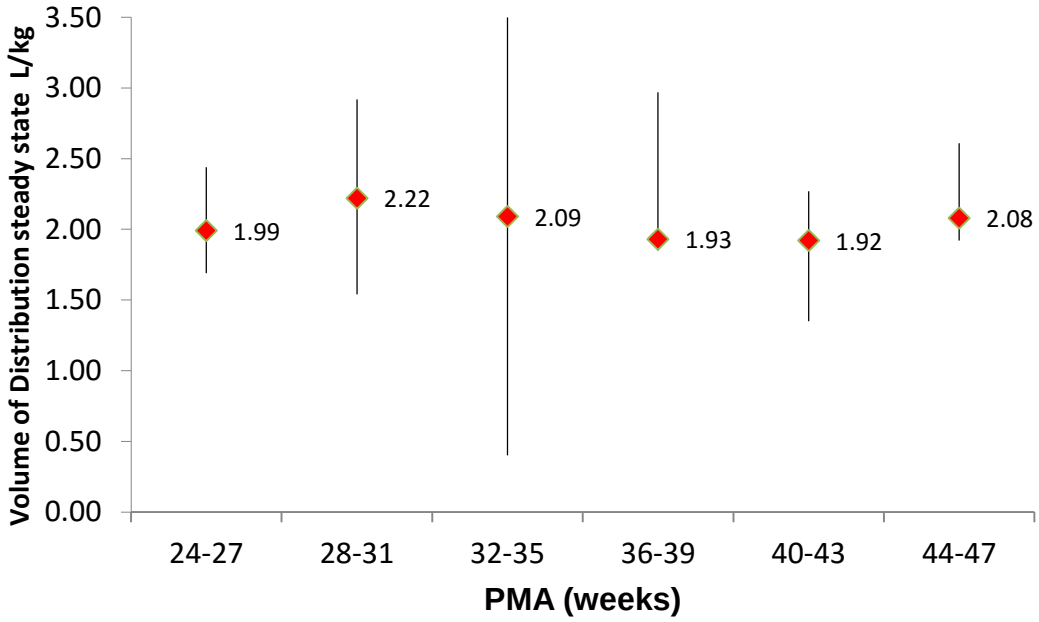



Figure 2-15 Volume of distribution (L/kg at steady state) and PMA Group
Median and range 2.20 (0.4 - 3.55) for PMA sub-age groups (n=60)

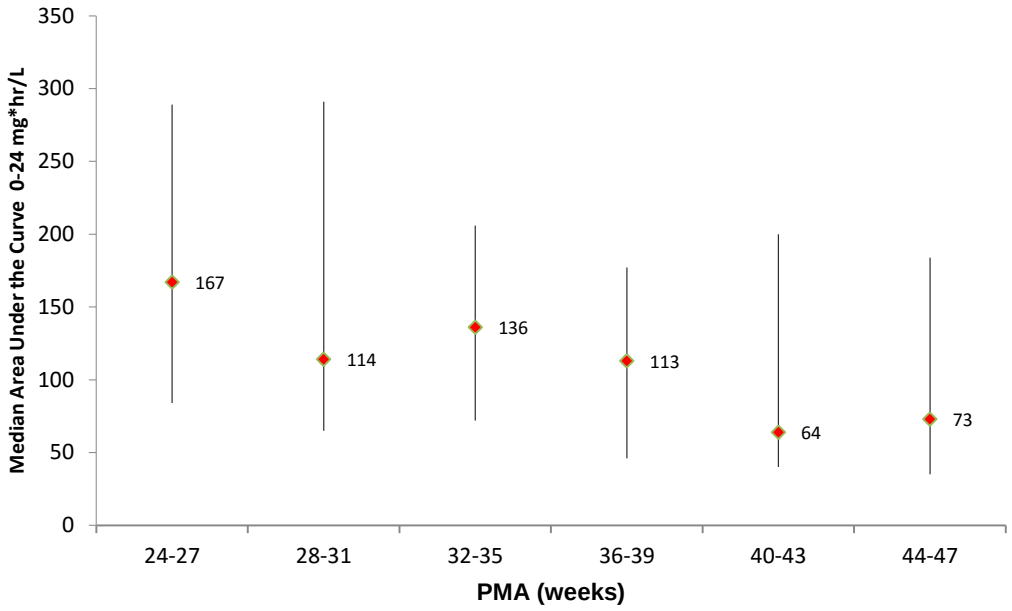



Figure 2-16 Area under the curve $_{0\text{-}24}$ and PMA Groups

The $AUC_{0\text{-}24}$ at steady state for each PMA groups (median and range) for the evaluated dose regimens ranged from 35 to 291 mg*h/L. (n=60)

Table 2-13 Co-efficient of variation (CV) % for PK Parameters

| PMA | Clearance (CL) % | Area under the curve (AUC) % | Half-life % |
|---|---|---|---|
| 24-27 | 60 | 44 | 58 |
| 28-31 | 35 | 46 | 48 |
| 32-35 | 66 | 33 | 37 |
| 36-39 | 44 | 34 | 40 |
| 40-43 | 38 | 53 | 41 |
| 44-48 | 65 | 50 | 31 |
| **Range** | 35-66 | 33-53 | 31-58 |

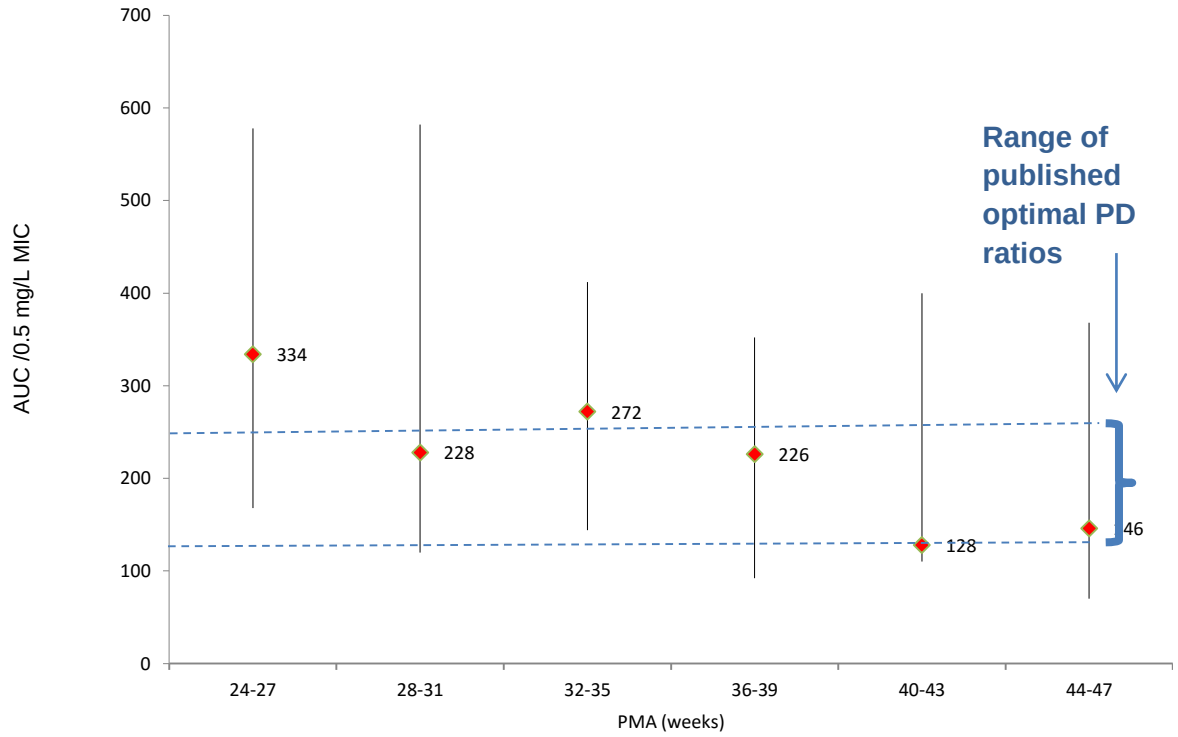



Figure 2-17 Area under the curve$_{0\text{-}24}$ and the Clinical Breakpoint

This figure illustrates the AUC at steady state/MIC 0.5 mg/L (median and range) representing each PMA Group based on EUCAST clinical breakpoint. The AUC ranged from 35 to 291 0-24 mg*h/L. The optimal pharmacodynamic predictor of clinical outcome is a minimum AUC/MIC ratio of 125 ranging to recommendations for target of 250.

A two compartment model with first order elimination showed the best fit with the data. The objective functional value (OFV) and residual variability of the two-compartment model were lower than a one compartment model. The model was parameterized in terms of central volume of distribution (V1), peripheral volume of distribution (V2), inter-compartment clearance (Q) and clearance (CL) of ciprofloxacin. Inter-individual variability was best described by an exponential model and was then estimated for V1, V2 and CL. Inter-occasion variability on CL was coupled to inter-individual variability by an additive model, respectively. A proportional model best described residual variability.

#### 2.3.3.2 Covariate analysis

Covariate analysis found that gestational age, postnatal age, current weight, serum creatinine and inotrope administration had a significant impact on ciprofloxacin PK parameters. The parameter estimates of the final PK model include the median (range) of estimated weight-normalized CL 0.20 (0.04-0.81) L/h/kg and volume distribution at steady-state (sum of $V_1$ and $V_2$) 2.02 (0.40-3.55) L/kg. The $AUC_{0-24}$ at steady-state for the evaluated dose regimens ranged from 35 to 291 mg*h/L. Ciprofloxacin CL increased allometrically with current weight, decreases with increasing creatinine concentration and showed a 29% decrease with co-administration of inotropic agents Table 2-14.

The allometric size approach was used by incorporating *a priori* the current weight into the basic model (allometric coefficients of 0.75 for CL and Q, 1 for V1 and V2), caused a significant drop in the OFV of 113.9 points from the structural model. Postmenstrual age was identified as the most important covariate on CL, associated with a drop in the OFV of 56.0 units. However, gestational and postnatal age together proved to be superior (ΔOFV 66.7 units) to postmenstrual age alone. A further decrease in the OFV of 25.8 units was achieved by implementing serum creatinine concentration on clearance. The model was further improved by introducing co-administration of inotropic agents (ΔOFV 7.5 units) as a third covariate on clearance. For the central volume of distribution V1, only the co-administration of inotropic agents caused a significant drop in the OFV of 3.9 points in the forward selection process. However, it was not retained into the model after the backward selection process. The covariates were tested for significance using the OFV and included in the model when significant p=<0.05 = >3.84 (p= <0.01 = OFV >6.6). An allometric model was used therefore the difference between OFV and each covariate was tested against current body weight. Ciprofloxacin clearance variability was explained by size 31.2%, renal maturation 25.6%, renal function 5.7% and co-administration of inotropic agents 2.4%.

#### 2.3.3.3 Model evaluation

The mean parameter estimates resulting from the bootstrap procedure closely agreed with the respective values from the final population model, indicating that the final model is stable and can re-determine the estimates of population PK parameters Figure 2-18Figure 2-19. Model diagnostics showed acceptable goodness-of-fit for the final model of ciprofloxacin. Predictions are unbiased.

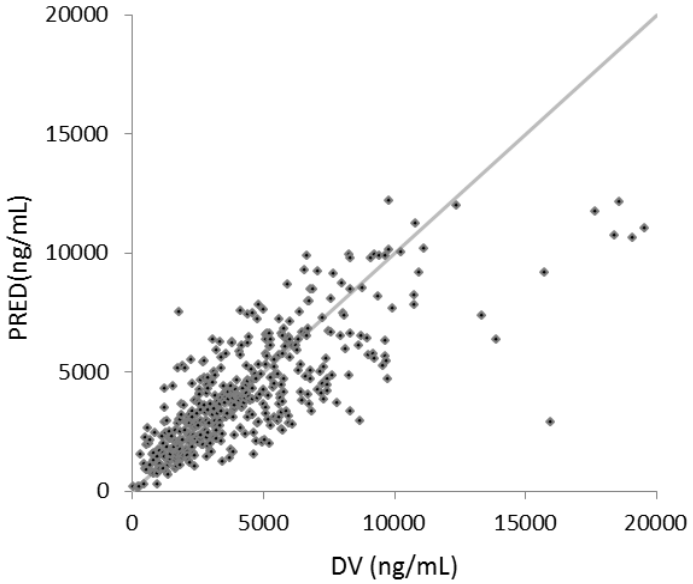



Figure 2-18 Predicted and actual concentrations of ciprofloxacin (direct variable ng/mL)

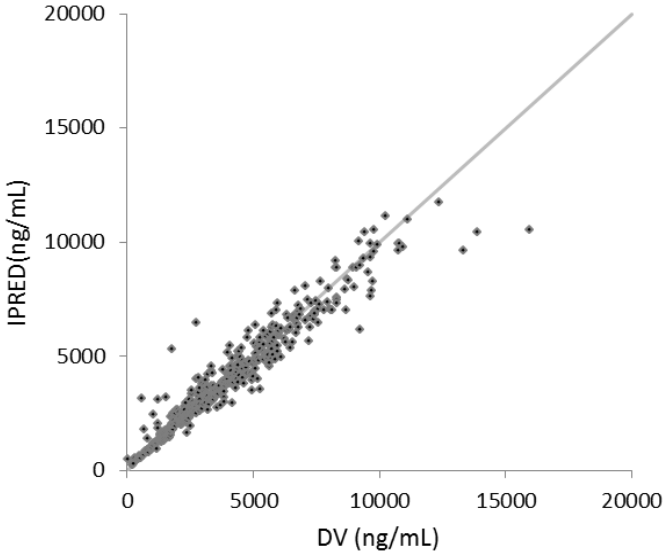



Figure 2-19 Individual predicted and actual concentrations ciprofloxacin (direct variable ng/mL)

Table 2-14 PK covariate analysis and Objective Function Value (OFV)

| **Structural Model**<br>**Allometric model** | **PK parameter**<br>**CL, $V_1$, $V_2$, Q** | **OFV**<br>**6569** | **P= 0.05 if OFV >3.84** |
|---|---|---|---|
| **Current body weight** | | **6455.1** | 113.9 |
| **Impact of age** | **V1** | | |
| GA | | 6454.9 | 0.2 |
| PNA | | 6451.9 | 3.2 |
| PMA | | 6453.2 | 1.9 |
| **Impact of age** | **V2** | | |
| GA | | 6455.1 | 0 |
| PNA | | 6452.3 | 2.8 |
| PMA | | 6453.7 | 1.4 |
| **Impact of renal maturation** | **CL** | | |
| Birth weight | | 6442.0 | 13.1 |
| GA | | 6438.7 | 16.4 |
| PNA | | 6425.2 | 29.9 |
| PMA | | 6399.5 | 55.6 |
| Birth weight and PNA | | 6396.2 | 58.9 |
| **GA and PNA** | | **6388.4** | 66.7 |
| **Impact of renal function** | **CL** | | |
| Serum creatinine | | **6429.3** | 25.8 |
| **Impact of renal maturation and renal function** | **CL** | | |
| **GA, PNA and serum creatinine** | | **6371.3** | 83.8 |
| **Impact of renal maturation, function and co-medication** | **CL** | | |
| GA, PNA, serum creatinine and diuretics | | 6371.4 | 83.7 |
| GA, PNA, serum creatinine and caffeine | | 6369.2 | 85.9 |
| GA, PNA, serum creatinine and teicoplanin | | 6371.0 | 84.1 |
| GA, PNA, serum creatinine and amoxicillin- clavulanic acid | | 6366.8 | 88.3 |
| GA, PNA, serum creatinine and nystatin | | 6370.9 | 84.2 |
| **GA, PNA, serum creatinine and inotropic agents** | | **6363.8** | 91.3 |

**GA:** gestational age; **PNA**: postnatal age. **PMA**: postmenstrual age.

Table 2-15 Population PK parameters of ciprofloxacin and bootstrap

| Parameters | Full dataset | | Bootstrap | |
|---|---|---|---|---|
| | **Final estimate** | **RSE (%)** | Median | **5th – 95th** |
| $V_1$ (L) | | | | |
| $V_1$=θ1× (CW/1955) | | | | |
| θ1 | 1.97 | 17.7 | 1.82 | 0.78 – 2.59 |
| $V_2$ (L) | | | | |
| $V_2$=θ2× (CW/1955) | | | | |
| θ2 | 1.93 | 21.9 | 1.97 | 1.38 – 3.02 |
| Q (L/h) | | | | |
| Q=θ3× $(CW/1955)^{0.75}$ | | | | |
| θ3 | 2.5 | 32.6 | 2.62 | 1.02 – 5.41 |
| CL( L/h ) | | | | |
| CL=θ4×$(CW/1955)^{0.75}$×$F_{age}$×RF×$F_{inotrope}$ | | | | |
| θ4 | 0.366 | 6.0 | 0.365 | 0.323 – 0.407 |
| $F_{age}$= $(GA/27.9)^{θ5}$×$(PNA/27)^{θ6}$ | | | | |
| θ5 | 2.11 | 11.9 | 2.09 | 1.60 – 2.57 |
| θ6 | 0.494 | 10.8 | 0.492 | 0.386 – 0.606 |
| RF= EXP((CREA-42)× θ7) | | | | |
| θ7 | -0.00335 | 46.0 | -0.00331 | -0.00753 – 0.00063 |
| $F_{inotrope}$ | | | | |
| θ8 | 0.708 | 10.9 | 0.719 | 0.572 – 0.869 |
| **Between Subject Variability (BSV) Inter-individual variability (%)** | | | | |
| $V_1$ | 48.1 | 63.6 | 49.6 | 26.2 – 77.2 |
| $V_2$ | 49.3 | 68.3 | 51.2 | 15.8 – 76.9 |
| CL | 33.2 | 19.9 | 31.3 | 25.3 – 37.4 |
| **Between Occasion Variability (BOV) Inter-occasion variability (%)** | | | | |
| CL | 16.4 | 55.6 | 16.6 | 9.2 – 26.2 |
| **Residual unexplained variability (RUV) (%)** | 19.3 | 28.2 | 18.7 | 14.8 – 23.1 |

**$V_1$**: central volume of distribution; **$V_2$**: peripheral volume of distribution; **Q**: inter-compartment clearance; **CL**: clearance; **RF**: renal function; **CW**: current weight in gram; **$F_{inotrope}$**: scaling factor applied for patients co-administrated with inotropic agents; **CREA:** serum creatinine concentration in μmol/L; GA: gestational age in weeks; PNA: postnatal age in days.

Table 2-16 Creatinine (µmol/L) levels and inotrope administration

| | Inotropes administered (n=20)* | No Inotropes Administered (n=36) | Difference 95% CI |
|---|---|---|---|
| Mean (St Dev) | 81.80 (42.58) | 40 (14.75) | |
| Median (IQR) | 74.50 | 37.5 | 36.5 |
| | (46.50 - 114.50) | (30- 44.5) | (18.0, 58.00) p=0.0001 |

Neonates (n= 56) were evaluated to compare creatinine levels at baseline for those administered inotropes. Those administered inotropes had significantly higher creatinine levels prior to the initial PK levels blood samples were collected. (n=56)

### 2.3.4 Cerebrospinal fluid concentrations

Concentrations in CSF samples (n=6) with exact collection times were obtained post administration of ciprofloxacin ranged from 187 to 1650 ng/mL, respectively Table 2-17. The median value of $CSF/_{serum}$ ratio was 0.32 (range 0.08 -0.58). A trend for correlation between CSF collection time and $CSF/_{serum}$ concentration ratios suggests less elimination from (or diffusion in) the CSF than the systemic circulation.

Assessment of the ciprofloxacin penetration into the cerebrospinal fluid (CSF) was evaluated by the CSF/serum ciprofloxacin concentration ratio. Because serum ciprofloxacin concentrations were not obtained concurrently with CSF sample collection, serum concentrations at the time of CSF sample collection were estimated via Bayesian estimation by the developed model.

Table 2-17 CSF concentrations and predicted serum concentration

| Patient ID | PMA (weeks) | Predicted serum concentrations (ng/mL) | CSF concentrations (ng/mL) | CSF/serum concentrations ratio |
|---|---|---|---|---|
| LW07 | 28.2 | 2830 | 1650 | 0.58 |
| LW20 | 30 | 2001 | 701 | 0.35 |
| LW21 | 36.7 | 2347 | 680 | 0.29 |
| LW35 | 27.2 | 4508 | 351 | 0.08 |
| AH24 | 41.5 | 471 | 187 | 0.40 |
| AH36 | 38.8 | 2403 | 686 | 0.29 |

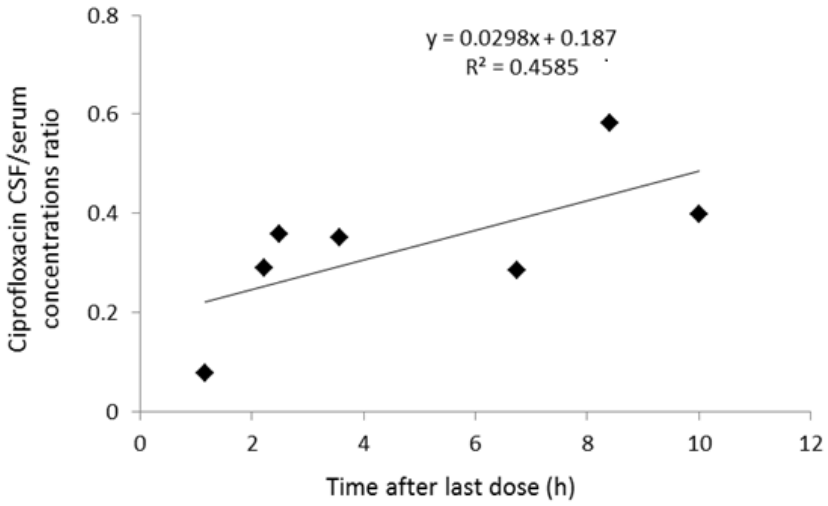


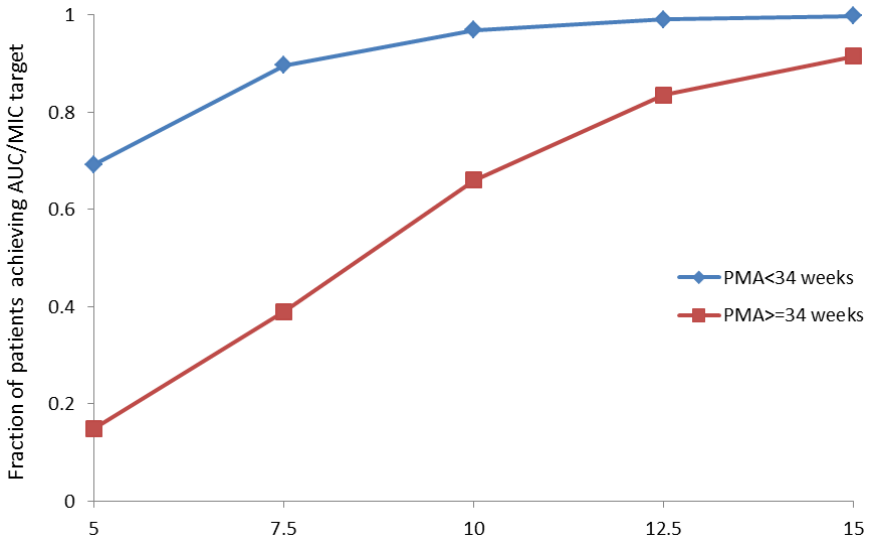

Figure 2-20 Ciprofloxacin CSF/serum concentrations ratio versus time (n=7)

### 2.3.5 Dose optimisation

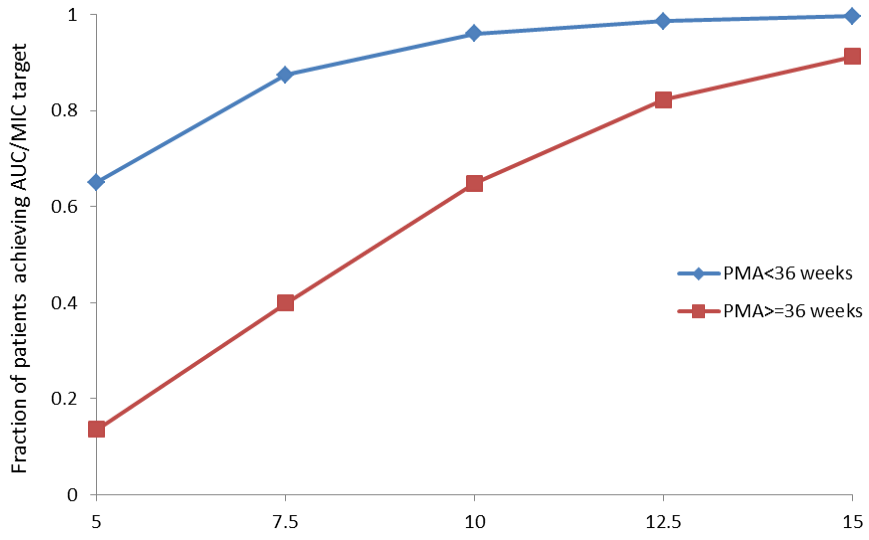
The target attainment rates as a function of dose and age groups were estimated for an AUC/MIC target of 125 using the EUCAST susceptibility clinical breakpoint of 0.5mg/L. Two visual cut-off points of age PMA 34 weeks and 36 weeks to represent nephrogenesis were evaluated as shown in Figure 2-21. Monte Carlo Simulation demonstrated that 90% of hypothetical new borns with PMA <34 weeks treated with 7.5 mg/kg twice daily and 84% of new borns with PMA ≥34 weeks and young infants receiving 12.5 mg/kg twice daily would reach the AUC/MIC target of 125 using the EUCAST susceptibility of 0.5mg/L.

The paediatric dose of ciprofloxacin was simulated on a mg/kg basis according to different age group for various mg/kg dosing regimens (5, 7.5, 10, 12.5, 15 mg/kg twice daily) Table 2-18. One thousand simulations were performed using the original dataset, and the $AUC_{0\text{-}24}$ at steady state was calculated for each simulated patient. The target attainment rate was then calculated for each dosing regimens to define the optimal dose regimens in each neonatal group.

a)




b)




Ciprofloxacin dose mg/kg/bi-daily

Figure 2-21 Dose v Target Attainment of the AUC/MIC 125 with age

For PMA 34 weeks and B) for PMA 36 weeks

Table 2-18 The percentage of neonates that achieve an AUC/MIC 125 ratio simulated for dose regimen (mg/kg) administered 12 hourly

This illustrates the dose of ciprofloxacin required to achieve the AUC/MIC target of 125 based on EUCASTs non-species specific clinical breakpoint (0.5mg/L). Two cut off points for PMA s were selected to allow a comparison of the age range between which nephrogenesis is estimated to occur a) PMA above and below 34 weeks b) PMA above and below 36 weeks.

(a) Comparing neonates pre and post completion of nephrogenesis when estimated as 34 weeks PMA

| PMA (weeks) | Dose (mg/kg) BID | | | | |
|---|---|---|---|---|---|
| | **5** | **7.5** | **10** | **12.5** | **15** |
| **<34** | 0.69 | 0.90 | 0.97 | 0.99 | 1.00 |
| **≥34** | 0.15 | 0.39 | 0.66 | 0.84 | 0.92 |

(b) Comparing neonates pre and post completion of nephrogenesis when estimated as 36 weeks PMA

| PMA (weeks) | Dose (mg/kg) BID | | | | |
|---|---|---|---|---|---|
| | **5** | **7.5** | **10** | **12.5** | **15** |
| **<36** | 0.65 | 0.87 | 0.96 | 0.99 | 1.00 |
| **≥36** | 0.14 | 0.40 | 0.65 | 0.82 | 0.91 |

### 2.3.6 Clinical outcome and Pharmacovigilance

The clinical test-of-cure cure defined cure as survival and CRP <10mg/L three days after the cessation of ciprofloxacin compared to treatment failure. This definition of cure was based on the fact that there are no standard clinical outcome measures for neonatal sepsis (see section 1.5.1.1). This approach is pragmatic reflecting clinical decision making the availability of CRP from clinically required blood tests. CRP data were available on day 10 for 39 neonates; 28/39 (71%) had CRP value <10 mg/L. The survival status or CRP data (3 days after the cessation of ciprofloxacin) were available for 61 participants: one participant was transferred to another hospital and lost to follow-up. Overall 47/60 (78%) were cured. Of those classed as cured, 28% did not have a CRP >10mg/L during the course of ciprofloxacin. Among the 48 with a CRP

>10mg/L during the course of ciprofloxacin 34 (71%) were cured. The four participants with proven Gram-negative infections were cured.

Table 2-19 Indication for ciprofloxacin treatment for suspected Gram-negative Sepsis (n=64)

Ciprofloxacin was prescribed for suspected Gram-negative sepsis and selected as opposed to other antimicrobials when indicated:

| **Indication** | **%** | **N=** |
|---|---|---|
| Second line following gentamicin | 80 | 51 |
| Renal sparing | 12.5 | 8 |
| Suspected meningitis | 1.5 | 1 |
| Proven meningitis | 1.5 | 1 |
| Proven blood culture | | |
| *Enterobacter cloacae* | 1.5 | 1 |
| *Haemophilus influenza* | 1.5 | 1 |
| *Serratia marcescens* | 1.5 | 1 |
| *Pseudomonas aeruginosa* | 1.5 | 1 |

### 2.3.7 Serious Adverse Events

As part of pharmacovigilance data safety monitoring reports as required by Ethics and the MHRA there were 24 Serious Adverse Events (SAE) reported to the Sponsor. Throughout the study there were no Suspected Unexpected Serious Adverse Reactions (SUSAR). In this critically ill population SAE were anticipated as complications associated with extreme prematurity and suspected Gram-negative sepsis Table 2-20. The independent causality assessments for each case were deemed to be unlikely in relation to ciprofloxacin administration. Eight deaths were reported during the six week pharmacovigilance period, a mortality rate of 13%. Four occurred within ten days of treatment with ciprofloxacin. 82% of these deaths were babies born very premature <26 weeks PMA. A further three babies died prior to discharge after the 42 day period for regulatory pharmacovigilance reporting, in total eleven (18%) (Table 2-21). The base-line mortality in this neonatal unit is 20% for those born <28 weeks PMA and 8% for those 28-34 weeks PMA.

Table 2-20 Potential Serious Adverse Events reported to the Sponsor (n=24)

| Potential SAE/SAR | | N= | Causality |
|---|---|---|---|
| No SAE reports<br>Potential SAE reports<br>SUSAR | | 41<br>24<br>0 | |
| Death | <28 weeks PMA (birth)<br>>28 weeks PMA (birth) | 7<br>1 | Probably not related<br>Definitely not related |
| Cardiac | Cardiac arrest | 2 | Probably not related |
| Hepatic | Deranged liver function | 1 | Possible |
| Multi system organ failure | | 1<br>1 | Probably not related<br>Definitely not related |
| Neurology | Hydrocephalus<br>Fitting | 1<br>1 | Definitely not related<br>Probably not related |
| Renal | Decreased renal function<br>Bilateral renal calculi<br>Haematuria<br>Echo genesis of medulla | 1<br>2<br>1<br>1 | <br><br>Probably not related<br> |
| Respiratory | Desaturation and bradycardia<br>Polyps (surgery) | | Probably not related<br>Definitely not related |

Table 2-21 Mortality and PMA at birth

| Time to death after starting ciprofloxacin (days) | PMA at birth (weeks) | | |
|---|---|---|---|
| | <26 | 27-31 | >32 |
| < 10 | 4 | 0 | 0 |
| 10 - 42 | 3 | 1 | 0 |
| > 42 | 2 | 1 | 0 |
| Total | 9 | 2 | 0 |

#### 2.3.7.1 Haematology and Biochemistry Values

Of those recruited 10% had a creatinine >100 µmol/L prior to commencing treatment classed as a surrogate for renal failure within the unit. 23% had a creatinine >58 (upper limit of the reference range). Creatinine was high in 2/61 (3.2%) with a median increase across the whole study population of 1micromol/L on therapy and a median reduction from baseline of 4 micro mol/L three days after the end of treatment. Bilirubin on-therapy was high in 19/55 (35%). On-therapy AST was high in 5/56 (8.9%) and ALT was high in 7/56 (12%) but both parameters were a median of 16 IU/L below the baseline value at 3 days after treatment was stopped.

Renal dysfunction was classed as an adverse event (possible nephrotoxicity) when *'serum creatinine doubled or increased >44 µmol/L over baseline for blood samples*

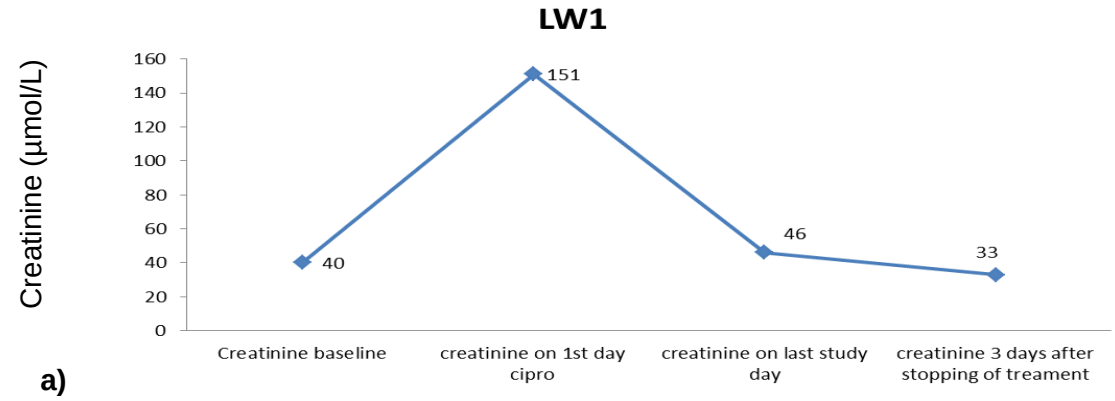
*taken prior to commencing ciprofloxacin '*. This was based on consensus of opinion of the TINN Consortium. The laboratory reference range was 30-58 µmol/L. Three recruits were assessed as possible nephrotoxicity but subsequently the blood were found to be taken earlier in the day prior to ciprofloxacin being commenced. Each had increased creatinine on the first day of ciprofloxacin Figure 2-22. LW1 and LW16 creatinine subsequently decreased during ciprofloxacin treatment and LW28 increased.

Table 2-22 Haematology and Biochemistry Values during Ciprofloxacin Therapy

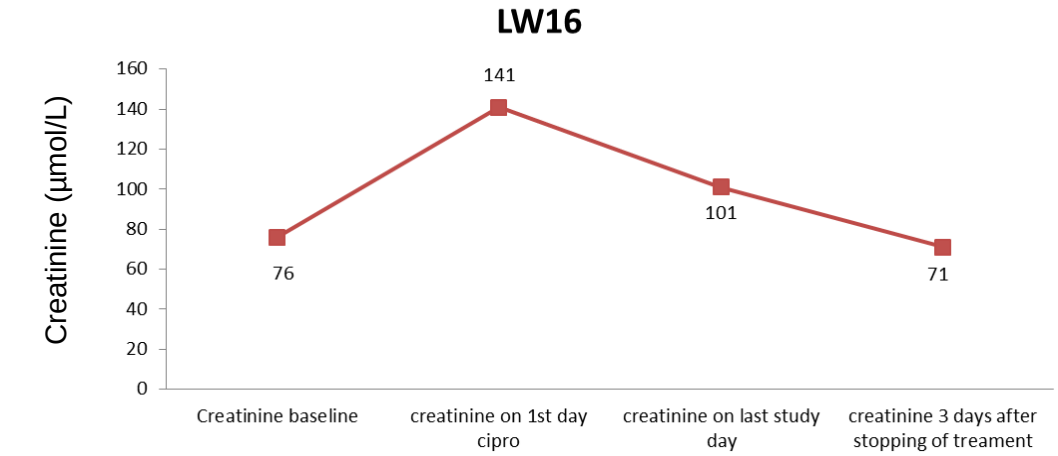
| **Blood value** | **Reference range** **(>14 days of birth)** | **Baseline median (IQR) n=** | **Range n =** | **% <normal >normal** |
|---|---|---|---|---|
| | | **n=58** | **Minimum on therapy n=61** | |
| Haemoglobin g/L | 13 - 21 | 11 (10-14) | 11 (9-12) | < 52 |
| Platelets | 150 - 400 | 198 (110-310) | 147(64-294) | < 51 |
| Haematocrit | 42 - 66 | 35 (30-40) | 30 (29-40) | < 61 |
| White cell count | 5 -18.4 | 14 (10-18.5) | 12 (10-18) | < 6.6 |
| Albumin g/L | 30 - 45 | 27 (25-30) | 25 (20-28) | < 87 |
| | | | **Maximum on therapy** | |
| Creatinine µmol/L | 30 - 58 | 43.5 (34-64) n=50 | 46 (36-68) n=61 | |
| Lactate mmol/L | 0.7 – 2.1 | 1.3 (1.1-2.1) n=48 | 1.8 (1.3-2.8) n=58 | |
| Magnesium mmol/L | 0.78 – 1.02 | 0.85 (0.75 -0.92) n=52 | 0.7 (0.67-0.81) n=62 | |
| Total Bilirubin µmol/L | <340 | 82 (25-245) n=46 | 57 (23-163) n=55 | >35 |
| AST IU/L | 23 – 73 | 37 (26-83) n=41 | 44 (28-85) n=56 | >8.9 |
| ALT IU/L | 9 - 44 | 27 (12-78) n=39 | 29 (17-65) n=56 | >12 |
| Alk phosphate IU/L | <1600 | 590 (341-1230) n=40 | 687 (469-956) n=56 | |

Table 2-23 Creatinine values µmol/L

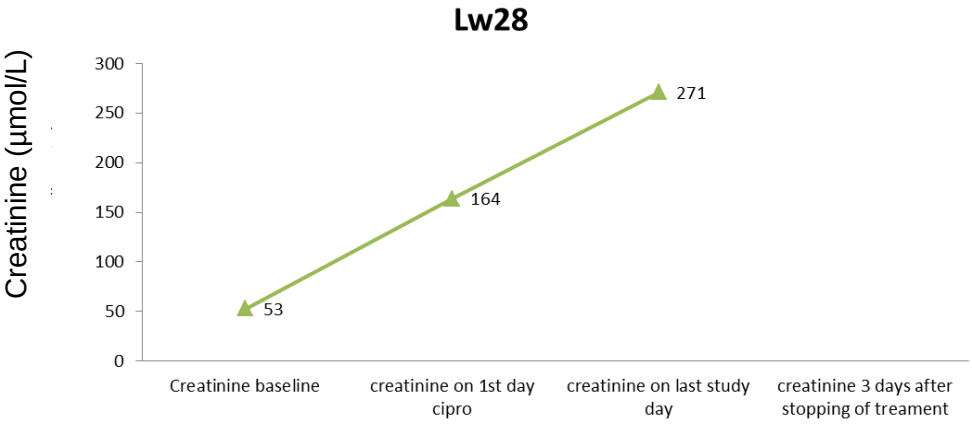
| **Serum creatinine concentration (µmol/L)** | **Pre-treatment baseline** | **Day 1 Pre or post dose** | **Last day of ciprofloxacin** |
|---|---|---|---|
| **N** | 57 | 61 | 40 |
| **Median** | 42 | 42 | 40 |
| **Range** | (26-188) | (22-232) | (24-271) |




a)




b)




c)

Figure 2-22 Increased creatinine (µmol/L) at baseline

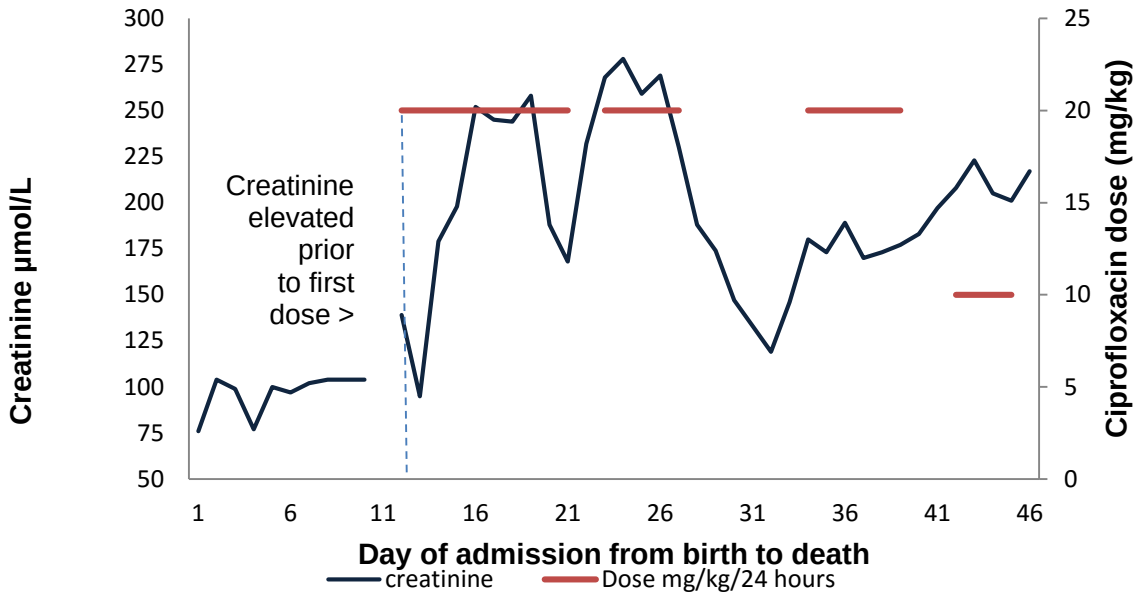



Figure 2-23 Creatinine and Ciprofloxacin Administration LW17

LW17 Creatinine concentrations were 139 on day 11 before commencing ciprofloxacin. On day 22 ciprofloxacin was recommenced but the creatinine was 232 before starting. On day 34 the creatinine was elevated prior to ciprofloxacin.

Two cases of renal calculi (renal stones) and nephrocalcinosis (calcification in the renal parenchyma) were classed as non-serious adverse events with possible causality. **LW02**: male born at 26 weeks PMA weighing 780g. **Clinical history:** Trisomy 21 with duodenal atresia and severe broncho pulmonary dysplasia. An episode of oliguria was associated with staphylococcal infection. A renal ultrasound showed several renal calculi bilaterally, the calculi were not present early in the admission and resolved at a later stage. Several courses of ciprofloxacin were given one before the normal scan, two between normal and abnormal scan and two between abnormal scan and recovering scan. **LW03:** male born at 28 weeks PMA weighing 1.17 kg. **Clinical history:** Anal stenosis then Meckel's diverticulum requiring an ileostomy. Haematuria noted on day 35. A renal ultrasound on day 39 showed both kidneys were normal in size but small calculi were evident bilaterally, more prominent on the right side (>4mm). There was no evidence of hydronephrosis or ureteric dilatation.

#### 2.3.7.2 Hepatotoxicity

Hepatotoxicity was defined as:

- Aspartate aminotransferase (AST) increased 10-fold over baseline
- Alanine aminotransferase (ALT) increased 10-fold over baseline
- Conjugated bilirubin>85.5 µmol/L
- Unconjugated bilirubin>256.5 µmol/L if <36 weeks PMA
- Unconjugated bilirubin > 342 µmol/L if ≥36 weeks PMA

Table 2-24 Laboratory Neonatal reference range for AST and ALT

| **AST/ALT** | **Post Menstrual Age** | |
|---|---|---|
| | **<26** | **>26** |
| **AST (units)** | **n=171** | **n=2434** |
| 10th centile | 24 | 19 |
| 50th centile | 64 | 33 |
| 90th centile | 674 | 115 |
| **ALT (units)** | **n=247** | **n=4004** |
| 10th centile | 2 | 6.6 |
| 50th centile | 11 | 11 |
| 90th centile | 129 | 47 |

Table 2-25 Liver Function Test Results Day 1 - 7

| **Liver Function** | **Base-line** | **Day** | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| **Bilirubin Conjugated (n)** | 15 | 13 | 10 | 6 | 8 | 8 | 6 | 4 |
| **Median** range (µmol/L) | 13 (4-128) | 11 (5-152) | 15 (2-152) | 12 (6-133) | 41 (7-160) | 93 (9-199) | 72 (4-139) | 18 (6-230) |
| **Unconjugated (n)** | 15 | 11 | 10 | 5 | 7 | 7 | 5 | 4 |
| **Median** range (µmol/L) | 69 (6-268) | 69 (7-245) | 46 (2-232) | 65 (4-157) | 45 (5-192) | 78 (4-137) | 36 (4-96) | 19 (5-83) |
| **AST** (n) | 41 | 31 | 29 | 26 | 20 | 19 | 12 | 12 |
| **Median** range (IU/L) | 37 (13-1023) | 41 (11-1734) | 35 (19-1041) | 34 (16-418) | 41 (18-169) | 40 (16-120) | 34 (19-98) | 33 (15-120) |
| **ALT** (n) | 39 | 31 | 29 | 26 | 20 | 19 | 12 | 12 |
| **Median** range (IU/L) | 27 (6-514) | 31 (10-463) | 29 (10-356) | 26 (7-322) | 20 (9-308) | 27 (11-227) | 34 (9-176) | 25 (11-81) |

Table 2-26 Highest values of conjugated bilirubin during ciprofloxacin treatment

| Patient ID LW | conjugated bilirubin (µmol/L) |
|---|---|
| 1 | 112 |
| 2 | 143 |
| 7 | 160 |
| 11 | 119 |
| 12 | 133 |
| 18 | 96 |
| 26 | 114 |

Seven patients had elevated conjugated bilirubin (reference range >85.5 µmol/L). The highest values of conjugated bilirubin for each of these patients during the ciprofloxacin treatment are shown in Table 2-26

Prior to starting ciprofloxacin LW18 had an elevated unconjugated bilirubin 268 µmol/L (reference range >256.5 µmol/L if <36 weeks PMA).

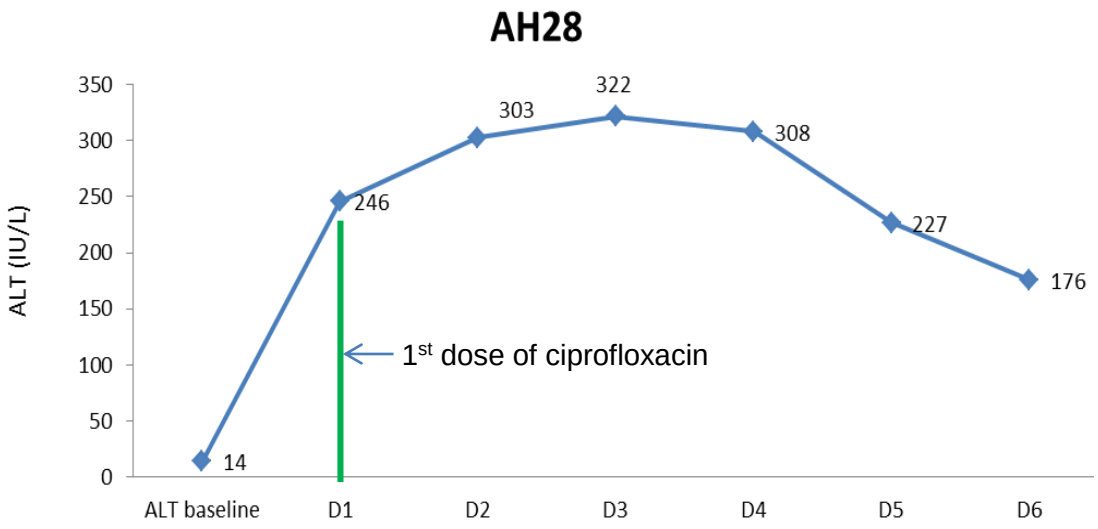



Figure 2-24 Hepatotoxicity AH28

**AH28:** Male 44 weeks PMA
**Clinical History –** cardiac surgery one day post bypass. ALT increased >10 fold. Changes in liver function were consistent with bypass.

### 2.3.7.3 Neurology

Seizures were reported in five participants prior to commencing ciprofloxacin. Clinical staff did not report seizures in participants after recruitment. Abnormal cranial

ultrasound findings were present at recruitment in 16 participants. One developed periventricular leucomalacia on cranial ultrasound after recruitment but this was not confirmed on magnetic resonance imaging.

#### 2.3.7.4 Arthropathy

There were no reports of arthralgia or arthropathy (altered mobility, pain and/or redness) in the follow up period up to six weeks.

#### 2.3.7.5 Tolerability of Ciprofloxacin

The reasons for discontinuing ciprofloxacin are shown in Table 2-27. The phlebitis score summarises the highest daily score

Table 2-28. The scale of 0- 5 assesses phlebitis by monitoring pain, redness, swelling and palpable venous cord. In total 259 daily assessments were made of infusions administered via peripheral lines (53%). Over 259 days only ten assessments (4%) reported either a 'possible' or 'early stage' phlebitis. 7.2% of the infusion sites scored 1-2 indicating possible early stage of phlebitis over this period. None of the participants had thrombophlebitis and seven required a line to be re-sited during treatment. One peripheral line was re-sited during an infusion

Table 2-27 Reasons for Discontinuing Ciprofloxacin Administration

| **Reason for discontinuing ciprofloxacin** | N |
|---|---|
| 5 day treatment schedule complete | 43 |
| Death during treatment | 3 |
| Resistant Organism | 0 |
| Symptoms resolved CRP<10 | 9 |
| Antimicrobial changed due to confirmed culture | 5 |
| Not tolerated | 0 |
| Total | 60 |

Table 2-28 Phlebitis Scores - tolerability of the intravenous infusion

| | **Days 1- 5** | | | | | **Total** |
|---|---|---|---|---|---|---|
| **Assessments** | **1** | **2** | **3** | **4** | **5** | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Neonates administered Ciprofloxacin | 62 | 62 | 51 | 44 | 40 | 259 |
| Central lines excluded | 27 | 28 | 24 | 23 | 19 | 121 |
| Peripheral lines | 35 | **34** | **27** | **21** | **21** | 138 |
| **Infusions administered over 30 mins** | **29** | **27** | **23** | **19** | **19** | **117** |
| **Phlebitis Score 1-5** | | | | | | |
| 1. Possible early stage | 1 | 1 | 1 | 1 | 0 | **4** |
| 2. Early Stage phlebitis | 0 | 1 | 2 | 1 | 1 | **5** |
| 3. Medium | 0 | 0 | 0 | 0 | 0 | **0** |
| 4. Advanced | 0 | 0 | 0 | 0 | 0 | **0** |
| 5. Thrombophlebitis | 0 | 0 | 0 | 0 | 0 | **0** |
| **Total** | **1** | **2** | **3** | **2** | **1** | **9** |
| **Infusions administered over 60 mins** | **6** | **7** | **4** | **2** | **2** | **21** |
| **Phlebitis Score 1-5** | | | | | | |
| 1. Possible early stage | 0 | 0 | 0 | 0 | 0 | **0** |
| 2. Early Stage phlebitis | 0 | 1 | 0 | 0 | 0 | **1** |
| 3. Medium | 0 | 0 | 0 | 0 | 0 | **0** |
| 4. Advanced | 0 | 0 | 0 | 0 | 0 | **0** |
| 5. Thrombophlebitis | 0 | 0 | 0 | 0 | 0 | **0** |
| **Total** | 0 | 1 | 0 | 0 | 0 | **1** |

## 2.4 Discussion

The strength of the population PK clinical trial is that critically ill neonates and young infants for each sub-age group were recruited. These data represent the variability of the target population treated with ciprofloxacin that influences parameters which is essential as these data and analyses will ultimately contribute to licensing ciprofloxacin for neonates and young infants. Each stage of development is represented including those born extremely premature as early as 23.3 weeks PMA at birth and young infants (median 30.4 range 23.3 – 42 weeks). In total 64 babies were recruited, with between seven and thirteen participants for each sub-age group representative of every four weeks of development. Extreme variation in concentrations is evident between subjects even within each individual sub-age group. For neonates 28-31 weeks PMA the $AUC_{0-24}$ (SD) ranged between 65 – 291 mg*h/L (60). The majority were recruited in intensive care with complex comorbidities and clinical interventions associated with critical illness and suspected sepsis. Most babies (91%) were ventilated and 37% received inotropes.

Although this was a pilot PK trial this study has produced relatively rich data for each participant. Despite the limitations in blood sampling, on average 7.3 samples per neonate were collected of which 38% were scavenged from clinical samples without any additional burden to the baby. Despite this relatively large recruitment target these data are insufficient for more detailed sub-group analyses as they are stratified into seven age groups. The time frame for this sampling strategy was challenging because PK blood samples were collected within three or ten minutes of pre-defined time points yet this was achieved even though the drug was prescribed at any time day or night. The population design schedules produced data at six time points with between 25 -35 samples on day one for each time (T4 combined with T6) distributed up to twelve hours following the infusion providing AUC data to predict the probability of clinical cure. Other trials of this vulnerable population recruited far fewer participants

(<24) and limited to peak and trough data [182, 183, 185, 187]. The main findings of these data were:

- Clearance increased with age. The combined effect of gestational age and post menstrual age had a greater effect on clearance. Creatinine was inversely associated with clearance and decreased with age.
- Inotrope administration was associated with reduced clearance associated with underlying renal compromise at baseline.
- The conventional PD target from adult data AUC/MIC ratio >125 was achieved by the median of each sub—age group for the regimens administered.
- Ciprofloxacin penetrated the CSF and achieved a ratio of 0.33 of the serum plasma concentrations.

### 2.4.1 Multiple dose PK parameters and covariate analysis

Post menstrual age (PMA) was the most important single covariate on clearance, but the combined effect of gestational age (on administration) and PMA (at birth) had a greater effect. Ciprofloxacin is mainly excreted renally therefore this higher clearance with maturation is consistent with completion of nephrogenesis estimated as occurring between 34-36 weeks PMA [123]. The model suggests the main change in clearance within the sub-age groups is on completion of nephrogenesis. Less variation was evident within the sub-age groups above or below this cut off. The mean clearance increased considerably above 35 weeks PMA from 0.09 to 0.13 l/h/kg to 0.20 to 0.33 l/h/kg. Similarly, Lipman et al. found minimal variation in clearance when comparing infants (aged 3 and 12 months) 0.56 L/h/kg (SD 1.40) with young children (1 – 5 years age) 0.53 L/h/kg (SD 1.22) [115]. Similarly, this trial suggests a dosing interval of 12 hours before 34 weeks PMA and 8 hourly following this period. Regimens specific to each sub-age groups may be required consistent with neonatal PK studies of gentamicin found that those <29 weeks PMA required a lower dose regimens [126].

The combined effect of PMA and PNA together had more effect on clearance (OFV 66.7 for PMA and PNA v PMA alone OFV 56.0). Although this trial recruited a relatively large number of patients (n=64) these data were divided into six sub-age groups. As few as nine babies represented those very premature <28 weeks PMA therefore further data is required. This suggests the renal maturation for those born very premature is delayed post-natally more so than would have been in utero. Estimating the optimal dose may therefore have to factor in prematurity at birth as well as corrected gestational age when being prescribed the drug. Very premature neonates tend to be hospitalised for long periods and factors associated with immaturity or other morbidity. Adjustment of doses for prematurity will be relevant to many treated for late on set sepsis.

As this clinical trial was a population PK design there is wide variability in developmental changes pre and post nephrogenesis and in respect of critical illness. For example 12.5 % were prescribed ciprofloxacin due to renal compromise (Table 2-19) and renal function has considerable effect on concentrations. As a result the C max ranged considerably from 0.45 -15.9 mg/L. However, the median 3.5 (IQR 2.1 – 5.4) Figure 2-11 was below Lipman et al [115] reported 6.97 (SD 1.48) for infants 3 months age and above that found by Aggarwal et al [185] for pre-term neonates for 2.6 (SD 0.4) mg/L. The wide range illustrates the need for caution regarding toxicity and the importance of covariates such as renal function when determining population regimen. This highlights the need to individualise regimen during critical illness and developmental changes.

Renal function indicated by serum creatinine concentrations was significantly and independently correlated with ciprofloxacin clearance. Many risk factors for renal impairment co-exist in this population and renal failure is frequently (26%) reported in sepsis [254]. A high proportion had renal compromise prior to being recruited as 13% had creatinine >100 µmol/L. Ciprofloxacin is often the drug of choice when there are

early signs of renal failure as gentamicin is known to be nephrotoxic. Also, ciprofloxacin is generally a second line rescue therapy administered at a later stage when multi-system organ failure may be increasingly evident. These factors account for high creatinine levels reported on day one. It is important to consider the time of the routine clinical bloods on day one as these were usually collected prior to the decision to commence ciprofloxacin as PK studies are comparing the effect of the body on the drug. A high creatinine on day one therefore was not adverse drug reactions. Creatinine concentrations correlated with clearance, suggesting it may be a useful biomarker when selecting the optimal regimens. This is practical as biochemistry values are readily available in critical illness. However, there are limitations, creatinine concentrations may be falsely low masking renal failure due to an increased volume of distribution following fluid resuscitation [254]. Creatinine may be less reliable as a marker for babies <5 days of birth due to the effect of maternal creatinine. Further data are required as these babies were excluded from this trial.

Inotrope administration resulted in decreased clearance by 29%.This is clinically relevant as 37% of this population were administered inotropes. In contrast clearance in adults increased when co-administered inotropes with antibiotics attributed to the increasing cardiac output and renal blood flow or a hyper-metabolic response to sepsis [105, 129]. Ceftriaxone clearance almost doubled in 30% of adults [91]. Similarly, vancomycin clearance increased in adults [129]; whereas a neonatal trial of the same drug found the opposite as clearance decreased by 28% with concurrent use of dopamine [255]. Further analysis of the ciprofloxacin PK data found the sub-group administered inotropes had increased median creatinine (difference 36.5 95% CI 18.0, 58.00 p=0.0001) prior to commencing ciprofloxacin

Table 2-16. Therefore inotrope administration is a marker of underlying renal failure. The different response between adults and neonates may be due to inadequate PK-PD data of inotropes in neonates compared to a more established evidence base for critically ill adults. The optimal dose of inotropes and optimal blood pressure for neonates particularly for each sub-age group is unknown. The limited PK/PD data that exists for inotropes in neonates has wide variation in plasma concentrations and poor correlation with blood pressure response [256-261]. The most common inotropes administered in this trial included dopamine (27%) and dobutamine (19%), there is uncertainty regarding the optimal regimens for different ages. A Cochrane review found dopamine achieved haemodynamic targets more than dobutamine (RR 0.41, 95% CI 0.25 to 0.65; RD -0.23, 95% CI -0.34 to -0.13; NNT = 4.3, 95% CI 2.9 to 7.7) [262]. Studies of dopamine in infants and older children suggested higher doses (>7.5 micrograms/kg/min) may be necessary to treat hypotension [263]. In contrast other uncontrolled studies in preterm infants found lower dose therapy (2 to 8 micrograms/kg/minute) may be more effective [264]. Equally, there is uncertainty amongst neonatologists about the acceptable lower limit for systemic arterial blood pressure [262]. This is compounded by limitations to monitoring blood pressure during late onset sepsis as umbilical arterial catheters tend to be removed early to reduce the risk of infection. Radial or femoral arterial lines are rarely used due to the risk of occluding blood supply to limbs resulting in amputation in rare cases. In summary, suboptimal renal perfusion appears to account for the reduced clearance when neonates were co-administered inotropes.

The main predictor of treatment success for ciprofloxacin is the $AUC_{24}$/MIC ratio. The median $AUC_{24}$/MIC for each sub-age group achieved the minimum conventional target 125. As many as 14% of subjects were below this target, they may have been

undertreated which is of great concern considering the increased morbidity and mortality associated with Gram-negative bacteraemia. Predicting the optimal dose is challenging as the AUC varies considerably ranging between 34 and 291mg/h/L at steady state Figure 2-16. Those above this optimal target may be exposed to adverse reactions. The PK Modelling simulated the optimal regimens to achieve the conventional $AUC_{24}$/MIC ratio of 125 for two age groups above and below: i) 34 weeks then ii) 36 weeks PMA. Neonates below 34 weeks PMA administered 20mg/kg/day were more likely to achieve the $AUC_{24}$/MIC ratio 125 (97%) compared to only 66% above this age. The proportions were similar when comparing those <36 weeks PMA as 96% achieve this target compared to only 65% of those >36 weeks PMA Table 2-18.

This clinical trial protocol aimed to evaluate $AUC_{24}$/MIC ratio 125 as this is the conventional target widely applied for adults over many years and as this study was cautious as the safety data for higher dose regime to target an AUC 250 for neonates was unknown. However, a higher regimen for neonatal bacteraemia is worthy of consideration due to the high mortality. However, there is compelling evidence from both paediatric and adult data to support a higher $AUC_{24}$/MIC ratio of 250 to improve outcome and reduce resistance [48, 115, 162, 176]. PD studies have shown that organisms are eradicated at an earlier stage when the AUC/MIC ratios are higher than 125 [162, 176]. Forrest *et al.* found bacteria were eradicated within 1.9 days when the ratio was 250 compared to 32 days below 125 ($p<.005$) [162, 265]. Zelenetski *et al.* found the risk of treatment failure was 27.8 times greater below an $AUC_{24}$/MIC 250 ($P=0.011$) [176]. Only 38% of the neonates in this trial achieved the higher $AUC_{24}$/MIC target of 250. The model did not predict the dose required to achieve an $AUC_{24}$/MIC >250. Further analysis of these data is planned to simulate the optimal dose to achieve the higher ratio. However, these data illustrate that the median of each sub-age group below 36 weeks PMA achieve a higher ratio than 250 ranging up to 578. Further safety data is required stratified to each sub age

group as the toxicity is unknown. Regimen may need to be adjusted for those born prematurely due to delayed renal maturation despite corrected gestational ages as illustrated by Vieux et al [127]. The TINN Consortium completed a safety study in new born mice and found no adverse effect of Ciprofloxacin at 10 and 30 mg/kg/day. However, a considerably higher dose than used in clinical practice of 100 mg/kg/day resulted delayed weight gain, impaired cardiorespiratory and psychomotor development and caused inflammatory infiltrates in the connective tissues surrounding the knee joint [266]. Toxicity is expected with antimicrobials when high dose regimen are administered therefore the optimal dose for the MPC and efficacy requires safety data.

The optimal interval between doses in the BNF C recommends either 12 hourly for neonates and 8 hourly for term babies this interval is supported by this trial's data. The median half-life was considerably longer ranging between for each sub-age group below 35 weeks PMA ranging between 9.10 -12.65 hours (max 33.8) compared with >35 weeks 4.9 – 7.10 (max 14). This is consistent with gentamicin, as the half-life averages approximately eight hours at a gestational age of 26 to 34 weeks compared with about 6.7 hours when 35 to 37 weeks gestation [173]. In adults the half-life increases in renal impairment and doses are adjusted based on renal function tests [267]. A systematic review of gentamicin for neonatal sepsis recommended that a once a day regimens may be superior in treating neonatal sepsis in neonates more than 32 weeks gestation [268]. In these ciprofloxacin data the half-life was considerably long in very premature neonates up to 33.8 hours. Caution is required if administering 8 or 12 hourly regimen that may result in toxicity. Further safety data is required to determine the optimal regimens. An extended dosing regimen of once in 36 to 48 hours may be more suitable for very preterm infants born less than 32 weeks gestation [269-271].

Of the 64 neonates recruited eight died within the six week pharmacovigilance reporting period and a further three prior to discharge (n=11). The majority of deaths 80% (n=9) were in those born <26 weeks PMA at birth. This mortality rate is lower than neonates with confirmed Gram-negative bacteraemia in this unit as only 6.3% of the trial participants had Gram-negative bacteria in blood cultures. Most were prescribed ciprofloxacin for suspected sepsis. Optimising the regimens for confirmed bacteraemia is particularly important as mortality in this unit is 20% if < 28 weeks PMA and 8% between 28-34 weeks but increases to 44% when Gram-negative bacteria are confirmed. Similarly, other neonatal studies reported mortality increased from 18 % to 36% with confirmed Gram-negative late onset sepsis [18]. A review of the long term consequence of prolonged sepsis found considerable effect on the long term quality of life as only 28% of neonates were alive and considered normal at 18 months follow up [30]. Early eradication of bacteria may be more important than the risk of some adverse reactions such as arthralgia that is known to resolve. Equally, adverse reactions must be considered relative to the risk associated with alternative antimicrobials such as gentamicin. Balancing the risk of higher doses with adverse reactions may depend on whether sepsis is suspected or confirmed as the risk of Gram-negative bacteraemia is greater. Rapid methods of identifying organisms would allow higher doses to be prescribed earlier.

The physiological changes associated with maturation and critical illness influence drug disposition associated with fluid shifts and hypoalbuminaemia [88, 89, 105, 162, 177, 272-274]. Wide variations in PK parameters were evident in this population. This was not reflected by changes in the volume of distribution. Gous *et al.* found the volume of distribution of vancomycin almost doubled early in sepsis on day two associated with aggressive fluid therapy in infants compared to day eight [275]. The volume of distribution for gentamicin on the second day of treatment was reported as 0.43± 0.12L/kg then decreased on the seventh day of treatment 0.29+-0.17 L/kg [104].

Such changes are less likely with drugs such as ciprofloxacin that are lipophilic as they have greater affinity for intracellular distribution. Roberts *et al.* found fluoroquinolones responded less to extracellular fluid changes than hydrophilic drugs (aminoglycosides, B Lactams, carbapenems and vancomycin) [86]. Equally, the change in volume of distribution of ciprofloxacin in adults with severe abdominal sepsis was not significant [105].

In critical illness PK parameters vary within a short time frame. Lipman et al found the Cmax was higher on day two than day one indicating the need for a loading dose [115]. This may be because the dose was insufficient to saturate the tissues during the initial distribution phase or that renal function deteriorated by the second day. When comparing the median of each sub-age group the volume of distribution was relatively constant. Loading doses are not commonly required for lipophilic drugs [88, 103]. Tayman et al. found the volume of distribution decreased during neonatal development for hydrophilic drugs but increased for fat soluble drugs [97]. Decreasing extracellular fluid and increasing body fat associated with maturation can influence concentrations. Further analysis of this trial's PK data to evaluate the changes in parameters between the early samples on days one or two and late samples between days five and seven would provide valuable insight to the variability pre and post suspected sepsis.

Changes in albumin levels are common in sepsis associated with capillary leak, poor nutrition and reduced production of albumin concentrations [86, 102]. As ciprofloxacin is moderately bound to protein 20-40% [53] hypoalbuminaemia may have a moderate effect on concentrations. Albumin was lower at baseline than the laboratory reference range (median 27 range 30-45g/L) Table 2-22. As many as 40% of septic patients are reported to have low albumin levels ≤25 g/dL (reference range 42g/L +-10%) [90]. Trans-capillary escape of albumin from the intravascular to extravascular space can increase considerably by 200% during the first two days of critical illness [90]. This

partly explains the vast increase seen in weight for septic neonates. The effect on PK parameters can be considerable; in relation to amikacin hypoalbuminaemia was associated with requiring a 1.6 fold higher loading dose [92]. Ceftazidime volume of distribution increased fourfold due to the increased extracellular volume associated with capillary leak [276]. Fluctuations in PK parameters are evident during the distribution and elimination phase may have been associated with hypoalbuminaemia. When the albumin is low a higher proportion of the drug is unbound within the extravascular compartment resulting in a high drug concentration [88, 89]. However, the unbound drug is then free to be eliminated resulting in a lower concentration [7, 84, 88]. As a result of critical illness PK concentrations fluctuate.

There is little published data relating to the later stage of sepsis when capillary leak resolves. In theory the drug distributed into the peripheral compartment during inflammation may return to the central compartment increasing drug concentration. This may result in an increase in the half life as the drug is redistributed from the tissues back into the central compartment [89]. Changes are evident clinically as capillary leak resolves and the weight decreases there is a vast increase in urine output in ml/kg. Fewer samples were available for the last day of treatment (58%) due to mortality or discontinuation of treatment. Further analysis of paired early and late sample data would provide useful information about the variability in concentrations over the different stages of sepsis. Less variability is expected with ciprofloxacin as it is lipophilic which this analysis may illustrate.

Extreme changes in weight are evident in this population over the sepsis period increasing up to 38% Figure 2 6. In paediatrics drug regimens are based on weight (mg/kg). To avoid over dosing the pre-sepsis weight is often used but may result in under treatment if the concentration of the drug is lower. This may be dependent on whether the drug is hydrophilic or lipophilic. These data may contribute to deciding

whether drugs should be prescribed on the pre-septic weight or adjusted daily based on the actual weight. The correct dose may change daily pre and post sepsis.

Therapeutic drug monitoring (TDM) may optimise therapy by responding to each phase of infection. However there are practical and ethical limitations to sampling and concentrations may change before laboratory results are available. A pragmatic approach is to estimate the optimal regimens initially using the significant co-variates (creatinine, post menstrual age at birth and post- natal age) found in these PK data. However, the wide range of PK parameters during critical illness per subject and per sampling occasion even within age sub-groups emphasise the need for an individualised therapy.

The risk of error with sampling and drug administration may have contributed to the variability in parameters. This risk is greater in neonates and young infants than adults due to the minute drug volumes, minute blood volumes and complex infusion lines. To ensure the reliability of data an extensive training programme for over 400 staff was implemented and the research fellow was on call seven days a week for the year. The interim analysis report included a higher concentration three hours after the drug was administered than three minutes after the infusion. Possible causes explored included:

i) delayed distribution between central and peripheral compartments
ii) delay in the drug entering the vein due to infusion line dead space
iii) inadequate flush volumes (particularly via central lines)
iv) syphoning of drugs from one line to another via a Y connection
v) poor peripheral perfusion at the capillary sample site
vi) contamination if sampling from the line in error where the drug had been infused (contrary to the standard operating procedure)
vii) laboratory measurement error

Different drug infusion practices existed at each hospital. Each site administered the infusion over a different time either 30 or 60 minutes, in the neonatal hospital this was 30 minutes therefore less risk to the data. Also, the paediatric hospital primed the

infusion line with saline first which resulted in up to 24 minutes delay between the drug leaving the pump and reaching the vein. By accurately recording the administration data this allowed adjustments for the delay to be estimated. The duration of an infusion is known to alter peak concentrations therefore may have affected values particularly the $C_{max}$ [277]. The flush may not have been adequate particularly for internal long lines such as Broviac lines as it is difficult to estimate the dead space. Flush volumes are kept to the absolute minimal. Post-operative cardiac infants are fluid restricted to as little as 1mL/kg/hour in total for all drugs and nutrition and are often administered as many as twenty drugs per day. Although, early PK values are valuable in an informative sampling schedule, a recommendation of the study is to delay the first blood sample time to ten minutes when drugs are administered via central lines. During critical illness infusions are administered simultaneously with multiple lines connected via a Y connector or traffic light. As fluids follow the route of least resistance the drug may enter another infusion line with less resistance then enter the vein at a later stage when the second infusion starts.

Variation in PK concentrations may have been influenced by different sampling practices. Most of these PK blood samples were collected from capillary heel pricks. Venous cannula samples were not collected due to the risk of contamination from the drug administered via the same line. Studies comparing blood gas results taken from capillary, venous and arterial sites found wide limits of agreement [278-280]. Poor perfusion is known to effect absorption of intramuscular or trans-dermal administration and is equally likely to affect sample concentrations [118].

The frequency of clinical sampling during critical illness provided a practical and ethical opportunity to scavenge blood that would otherwise be discarded. The PK sampling schedule was selected to be in line with the time clinical bloods were required for standard care. On average 2.8 additional samples were scavenged without additional burden to the baby. This opportunistic method of scavenging

samples was dependent on the precision of data provided by training staff providing 24/7 care. The scavenged samples gave similar PK models to the samples obtained at informative time points [273]. The data was reliable as the range of PK timed samples concentrations 52 to 10,961 (median 4066; 25th-75th: 2212-5748) ng/mL and scavenged samples 450 to 15,976 (median 3505; 25th-75th: 2191-5428). The limitation of this methods is that the scavenged bloods were clustered at the start of the day taken during a phlebotomist ward round therefore underrepresents the range of times required for AUC data. Also, the number of samples per patient included in the model varied >10 and is biased to more severely ill who require more clinical bloods.

There are considerable risks to data reliability when dealing with minute volumes of drugs and blood samples. Standards for conducting PK trials in neonates are required to minimise the risk to data reliability as the large neonatal PK studies are rarely repeated and can influence prescribing practice internationally. A recommendation of this study would be to develop international standards for neonatal PK with a view to accredited PK ready clinical wards and laboratories. This would be supported by training the clinical teams and implementing systems to achieve the precision required. Implementing changes to standard practice such as drug prescription charts that include the time the drug was given rather than a signature against the time prescribed would improve precision. Standardised case report forms with minimum data sets would ensure covariates are systematically collected such as fluid therapy, poly pharmacy and organ support that influence PK parameters. Data should be explored by clinicians before data lock due to the complexity of the population.

### 2.4.2 Ciprofloxacin penetration in cerebrospinal fluid (CSF)

Ciprofloxacin has good tissue penetration therefore maybe useful for treating meningitis. Obtaining CSF samples was particularly challenging as samples were only collected if a lumbar puncture was required for clinical care which had generally been

completed prior to ciprofloxacin administration as it is second line therapy). Despite these challenges samples were collected and immediately frozen for 10% of recruits. CSF drug concentrations in infants are often higher than children and young adults associated with changes in the blood brain barrier [111]. Correlation between the CSF collection time and CSF/serum concentration ratios suggests slower elimination from or diffusion into the CSF compared to the systemic circulation and the median value of $CSF/_{serum}$ ratio was 0.32 (range 0.08 -0.58). This is consistent with adult studies $AUC/_{CSF}$ v AUC/serum steady state ratio was reported as 0.24 – 0.43 which increase to 0.92 when meninges were inflamed [111, 113]. Whether levels are higher when meninges are inflamed is not evident from these data as only one patient had confirmed meningitis. Meningitis may be underdiagnosed as lumbar punctures are not always obtained prior to commencing antimicrobial therapy. Six patients had seizures prior to ciprofloxacin administration but there were no reports following treatment. There is a higher risk of toxicity and seizures have been reported with higher Cmax in children [115]. The recent systematic review on safety of ciprofloxacin excluded reports of seizures [190].

### 2.4.3 Characteristics of the population and short term outcome

This population had complex comorbidity and a high risk of mortality associated with extreme prematurity and critical illness confounding the outcome assessment in relation to ciprofloxacin. This is evident as most were recruited in intensive care, 91% were ventilated and 40% were co-administered inotropes during their admission. CRP data were available on day ten for 39 neonates; 28/39 (71%) had CRP value <10 mg/L classed as cure. Few participants (6.3%) were subsequently diagnosed with confirmed Gram-negative bacteraemia limiting the clinical outcome assessment. Suspected sepsis was more commonly diagnosed in males (62%) Table 2-11, this gender bias is consistent with other reports of infection but there is no consensus as to the cause to date. Also, ciprofloxacin is generally prescribed late in the course of

illness as a second line rescue therapy therefore this group are often deteriorating with unknown aetiology. These data are limited as the response to treatment may have been influenced by earlier antimicrobial therapy or co-administration of co-amoxiclav. This PK trial design aims to detect the effect of the body on a drug concentration therefore it was not designed or powered to evaluate safety or the clinical outcome of late on set sepsis.

#### 2.4.3.1 Short term safety and tolerability

There were no SUSAR reported during this period, due to the nature of this population 24 SAE were reported to the Sponsor and found to be unrelated to ciprofloxacin.

#### 2.4.3.2 Mortality

Eight babies died during the six week post dose pharmacovigilance reporting period and a further 3 prior to discharge (n=11). The majority of deaths were associated with extreme prematurity as 67% occurred in those aged <28 weeks PMA. The overall mortality was slightly lower than this unit's mortality rate of 20% for this age group <28 weeks PMA which decreases to 8% between 28-34 PMA weeks (LWH BADGER patient data system). Mortality generally increases with severe infection but the majority of babies in this trial were diagnosed with suspected rather than confirmed Gram-negative sepsis. The DMC were satisfied that the mortality rate was no higher than anticipated in this patient population.

#### 2.4.3.3 Arthropathy

Visual signs such as redness or swelling of joints were not reported. Arthropathy or arthralgia may have been underreported as there is no definitive method for diagnosing this in neonates. This population are not weight bearing and their response to handling may be a simple startle reflex rather than pain. This clinical limitation is evident in the systematic review of safety in children that did not include any reporting for infants less than six months age [190]. Even before the introduction of

fluoroquinolones there were reports of arthralgia during sepsis. Children often present with joint complaints including 18% orthopaedic disorders, 17.6 % autoimmune disorders, 19.6% joint complaints related to bacterial infection and 44% unknown [197]. Also, children with cystic fibrosis occasionally present with articular damage unrelated to quinolones [281, 282]. The WHO Selection and Use of Essential Medicines Report stated that arthropathy was found to be reversible, without long term sequelae and not convincingly correlated with the use of fluoroquinolones in children [226].

Ciprofloxacin is reported as being the least arthrotoxic of the quinolones. Chalmeau *et al.* found musculoskeletal events tended to be more frequent with Pefloxacin 18.2% versus 3.3%; for ciprofloxacin (P <0.06) [283].The mechanism of arthropathy is unknown, but may be associated with magnesium deficiency. Magnesium supplementation in immature rats reduced ciprofloxacin-induced chondrotoxicity [284]. The range of magnesium concentrations for this population reduced over time but was not considerably lower than the reference range Table 2-22. Caution is required when intravenous supplementation is administered as Stahlmann *et al.* found that co-administration with ciprofloxacin considerably reduce the AUC from 8.2 to 0.7 [205]. A review of arthralgia specific to blinded randomised controlled trials would be valuable. The systematic review of safety included 15/105 RCT but did not publish this data separately [190].

#### 2.4.3.4 Renal and Hepatic Function

Renal and hepatic function varied over the course of sepsis but no causality was associated with ciprofloxacin. In general creatinine median concentrations decreased over the period of administration. Three patients creatinine levels met the criteria for safety reporting (serum creatinine doubled or increased > 44 µmol/L over baseline). In each case the clinical biochemistry samples were collected prior to commencing

ciprofloxacin. The elevated creatinine led to ciprofloxacin being prescribed due to concerns for nephrotoxicity associated gentamicin. Figure 2-22

One case of renal calculi (renal stones) and one of nephrocalcinosis (calcification in in the in the renal parenchyma) were found on clinical abdominal scans. Crystalurea has been reported in pre-clinical studies [53] therefore the IDSMC were informed for further investigation of causality . Other factors known to be associated with renal calculi are common in this population. Short *et al.* found, 21 (27%) neonates had some form of renal calcification and four (5.1%) had renal calculi and the risk increased by 62% with oxygen therapy [285]. Renal calcification is a known complication of long term furosemide therapy, Toffolo *et al.* [286] reported 12 (63%) premature neonates with bronchopulmonary dysplasia had some sort of renal calcification including three (16%) with renal calculi that did not receive furosemide. Nephrocalcinosis was not subsequently associated with long term renal dysfunction at four to five years old [287]. A safety review of ciprofloxacin did not report nephrotoxicity or crystalluria during phase II and II trials [190]. Abdominal scans required for clinical care were reviewed retrospectively and the incidence was no higher than in the literature. The DMC were satisfied that there were no concerns regarding ciprofloxacin but recommended routinely scanning neonates in future studies.

Liver function tests were high in some babies but there was no change in median values following the start of ciprofloxacin and values were lower than baseline three days after the end of therapy. Hepatotoxicity was reported for a patient following emergency cardiac surgery. Ciprofloxacin was started the day after the initial rise and settled after a few days in keeping with post bypass alteration in LFTs. The start of ciprofloxacin treatment was associated with a statistically significant fall in the platelet count. Again, this could also be part of the natural history of infection. These data

suggests there was no temporal association between ciprofloxacin and altered liver function but are not conclusive.

#### 2.4.3.5 Phlebitis

Neonatal veins were assessed following the administration of ciprofloxacin for a total of 259 patient days and only 4% reported as ‘possible’ or ‘early stage’ phlebitis. This incidence is low and several drugs were often administered to the same vein in most cases. The BNF C recommends administration over 60 minutes as this injectable solution is acidotic (pH 3.5 – 4.6). This is longer than most antibiotic infusions in clinical practice and can be difficult as there are a limited number of lines when other drugs or total parenteral nutrition need to be administered. It was not possible to compare the effect of the short versus long infusion as most of the term babies had central lines. Ciprofloxacin was administered over 30 minutes to neonates suggesting the shorter infusion was tolerated even in the very premature.

In general ciprofloxacin was tolerated by this population. There was no clear, novel safety signal or evidence of unanticipated clinical events, acute articular problems, excess phlebitis or clinically significant derangements in laboratory parameters that could be attributed to ciprofloxacin. The reasons for stopping ciprofloxacin included death (unrelated), discontinuation of antimicrobial therapy or confirmed microbiology. As, three different regimens were administered to this population ranging between 10 – 30 mg/kg/day the outcome assessment is limited. PK data generated within a critical care population may not be generalizable to the wider paediatric population as other interventions may mask adverse events. These safety data included follow up data limited to six weeks. Neonates are more susceptible to drug related developmental disorders and adverse drug reactions can sometimes be detected later in life [79]. Further safety follow up date is required.

### 2.4.4 Recruitment, Ethical and Governance Challenges

The key to the success of the trial was developing a research culture with the wider clinical team and families. Ciprofloxacin was infrequently prescribed as it is a second line therapy. To avoid missing eligible participants over 2500 babies were screened daily each day of their admission. The screening log was cross checked against pharmacy records to confirm that no eligible babies were missed during this period. A high recruitment rate of 63% was achieved at the neonatal hospital and 43% at the children's hospital. By training the wider clinical team meant they were informed and could respond to questions from parents. This transparency reassured families who viewed the trial as being led by the clinical team.

The time frame for consent was challenging as the decision to prescribe ciprofloxacin could be anytime day or night. Administration had to commence within 30 minutes of the decision to prescribe the drug due to the risk of severe sepsis. Most babies were extremely ill; despite being in a stressful situation the parents accepted the importance of the research and had an altruistic approach to protect future babies. Prospective consent allowed families more time to consider participation and to be approached at a less stressful time before signs of sepsis. The population PK models allows some flexibility in sampling times, parents were reassured when the researcher selected a sampling schedule most in line with the blood gas or daily biochemistry bloods to minimise additional distress to their baby. Only one parent withdrew.

Neonates often move from a lead centre to a local neonatal unit during their care. There were only two recruiting sites but forty hospitals were set up with a Principal Investigator and R&D approvals at all units within 100 miles to provide follow on data. Only three babies were subsequently transferred and there were no safety issues reported. A recommendation of this trial is to promote a more proportionate approach to the governance requirements in Chapter 5.

### 2.4.5 Conclusion

Pharmacodynamic data and safety data specific to neonates and young infants is essential to apply PK parameters to determine the optimal regimen. This study has demonstrated that the regimens 10 mg/kg 12 hourly achieved the minimum acceptable PD target 125 ($AUC_{24}$/MIC ratio). Due to the severity of illness in Gram-negative sepsis a higher ratio may optimise outcome. It is evident from these PK data that the time interval between doses needs to adjust for completion of nephrogenesis. This is consistent with current recommendations for 12 hourly for preterm babies increasing to 8 hourly for those > either 34 or 36 weeks PMA [173]. Further individual dose adjustments are required for those born very premature as their renal function may mature at a slower rate post-natally. Also, for those with signs of renal failure are present indicated by raised creatinine and inotrope administration. In the absence of neonatal PD data the optimal dose remains unknown.

It is evident from the wide range in PK parameters that the clinical condition, clinical interventions and co-administration of other medication all influence PK parameters in addition to the dynamic changes within days or weeks in development. This strengthens the argument for individualised regimens in this population including timely adjustment of antibacterial dosing with real time application of therapeutic drug monitoring. Even with sub-age groups wide variability is evident to the extent there is a considerable risk of undertreating or overdosing. The optimal dose may change at each dosing interval pre and post sepsis due to the inter occasion variability. There are limitations to individualised dosing in neonates particularly due to restrictions on blood sampling yet this study provides data regarding the optimal parameters including PMA, PNA and creatinine to individualise regimens. This may improve both the clinical outcome and minimise antibacterial resistance. Equally, the interpretation

of data from PK studies in critical illness may not be generalizable to other populations due to these complex variables.

High mortality, morbidity and alterations in vital signs or clinical parameters outside the normal range are anticipated in this population, causality was not associated with the administration of ciprofloxacin. There was no evidence of arthralgia although the assessment is limited by a non-verbal and non-weight bearing population. To minimise the effects of confounding factors a blinded randomised controlled trial would be required for definitive safety or outcome analysis.

Despite the ethical, regulatory and logistical challenges of PK trials in neonates this study succeeded to recruit 63 neonates and develop methods to minimise the burden to babies or their families. Also, methods to achieve the precision are essential for reliable data in large clinical teams. In addition Chapter 3 describes methods for scavenging DNA for pharmacogenomic analysis and Chapter 5 a more proportionate regulatory model for pharmacokinetic clinical trials in this population.

# Chapter 3 Neonatal DNA Sampling

## 3.1 Overview

This sub-study aimed to evaluate whether donor blood transfusions interferes with genotyping in the short term. The DNA from the scavenged blood and the buccal samples were extracted for comparison of the genotype. Pharmacogenomic analysis for ciprofloxacin is not part of this study but will be undertaken the by the Pharmacogenomics Laboratory University Ulm, Germany

### 3.1.1 Aim and Objectives

**Aim:**

- To optimize the method of collecting DNA samples from neonates for future pharmacogenomic analysis.

**Objectives:**

- To compare the quantity and quality of DNA extracted from neonatal DNA in blood and buccal samples
- To determine whether DNA scavenged from clinically required full blood counts is contaminated by donor blood following allogenic transfusions.

## 3.2 Methods

DNA samples from a sub-group of neonates from the PK clinical trial were collected The DNA scavenged from a clinically required blood sample was compared with the buccal sample as the control. A sub-group of neonates were transfused at least one blood product including packed red cells (SAGM), platelets or fresh frozen plasma within a week of DNA sampling. Their transfusion history was recorded including the number, date and type of allogeneic blood products transfused. The time period between transfusion and DNA sampling was recorded. In addition, it was noted whether the blood product was irradiated or treated with methylene blue as this diminishes the white cells [288]. A minimum volume of 5µg DNA was required by the pharmacogenomic team. In neonates the yield from buccal cells was likely to be much lower (estimated as ~ 2 µg).To increase the yield further DNA was obtained from pooled scavenged full blood count samples taken for clinical care.

### 3.2.1 Inclusion criteria for the quality and quantity of DNA:

- Parental consent for neonatal DNA samples
- Recruited to the PK clinical trial
- **Inclusion for comparing contamination by a donor**
- Neonates transfused packed red cells (SAGM), platelets or fresh frozen plasma within a week of a clinical blood sample.

### 3.2.2 DNA Sample Collection:

#### 3.2.2.1 Buccal Samples

Cells were collected by gently scraping the inner cheek using an Isohelix SK-2 buccal swab kit (Kent UK). The product information states this will yield 1 to 10µg in adults http://www.isohelix.com/PDF/isohelix swab_brochure.pdf#page=2 (accessed March 2013).

#### 3.2.2.2 Scavenged Blood Samples

All blood samples collected for clinically required full blood count analysis were scavenged from the haematology laboratory in EDTA bottles over a period of 3 – 5 days. The samples were centrifuged at 4000 rpm for 10 min at 4°C to separate plasma components; the remaining cells and buffy coat were retained and stored cool.

### 3.2.3 DNA Extraction

DNA was extracted from buccal and blood samples by the Regional Molecular Genetics Laboratory at Liverpool Women's NHS FT. The method of extraction depended on the quantity of blood, when <700 µl was available a Qiagen kit EZ1 (Qiagen Inc, Manchester, UK) was used but for larger volumes >700µl Chemagen (Perkin-Elmer Beaconsfield, UK) extraction was performed according to the manufacturer's instructions. The extracted DNA was then stored at -40$^0$C until analysis.

DNA samples were anonymised and couriered on cool packs to the Pharmacogenomics Laboratory University of Ulm (Germany). The quantity sent was calculated using the formula: volume of DNA (µl) = 5000/DNA concentration (ng/µl) to ensure a minimum of 5 µg. Where insufficient DNA was available the whole sample was sent.

### 3.2.4 Quality of DNA Samples

To measure DNA purity and yield, each sample was analysed using a Nanodrop ND-1000 Spectrophotometer (version 3.3 Nanodrop Technologies Inc. Wilmington USA). DNA concentration is measured by absorbance at 260nm adjusted for turbidity measured by absorbance at 320nm http://www.promega.con/pubhub (May 2014). DNA purity absorbance was measured from a spectrum of readings between 230 – 320nm that detects the range of possible contaminants. DNA may be contaminated

by other molecules; RNA also absorbs at 260nm, Aromatic Amino Acids at 280nm and presence of guanidine increases A260, when present these may overestimate the DNA quantity.

Turbidity is indicated by high absorbance at 320nm. A strong absorbance at 230nm can indicate organic compounds or chaotropic salts are present in the purified DNA carried over from the DNA extraction procedure. This salt carryover can be evaluated by the ratio of A260nm to A230nm, the lower the ratio the greater the amount of salt present. The A260 /A230 ratio is best if greater than 1.5. The most common purity calculation is the ratio of absorbance at 260nm divided by 280 nm. Good quality DNA will have an A260/A280 ratio 1.7 – 2.0.

Table 3-1 DNA Concentration, yield and purity values

| DNA Measure | Range |
|---|---|
| **Concentration µg/mL** | (A260 reading – A230 reading) x dilution factor x 50 µg/mL<br>A260 of 1.0 = 50 µg/mL pure DNA |
| **Yield µg** | Concentration x total sample volume (mL) |
| **Purity (A260 / A280)** | (A260 reading – A320 reading) / (A280 reading – A320 reading)<br>Salt carry over: A260nm:A230nm<br>Good quality purity ratio 1.7 – 2.0<br>Optimal >1.5 a lower ratio suggests contamination or salt carry over |

### 3.2.5 DNA Analysis by PCR Typing

DNA PCR Typing was undertaken by the Regional Molecular Genetics Laboratory at Liverpool Women's NHS FT. The PowerPlex 16 HS System (Promega Madison USA) and GeneMapper Software (3.7) established the genotype. A comparison of the genetic profiles from both buccal and blood samples was made to determine the presence of peaks and the area under the peak (GeneMapper software version 3.7). This determines whether there was a match between buccal and blood cells or contamination. PCR allows for the simultaneous single-tube amplification and two-colour detection of eight polymorphic STR loci. CSF1PO, TPOX, TH01 and vWA primers are labelled with tetramethylrhodamine; D16S539, D7S820, D13S317 and D5S818 primers are labelled with fluorescein. Loci are named by two conventions. Some are named for nearby genes – such as THO1 (HUMTHO1, human tyrosine hydroxylase gene), while others, such as D7S820, are named for their chromosomal positioning. All of the loci included in this set are true tetranucleotide repeats. The quality control method was consistent with Promagen Standards that control changes in buffers, ionic strength and primer concentrations.

**Analysis for reporting:**

The percentage of contamination, where present, was quantified as:

Area of contaminating peak / Area of true peak x 100

The Genemapper software allocates allele designators (shaded blocks on electropherograms) that indicate where each allele should be compared to peaks on the electropherogram. The software size marker gives the size of the allele and calculates the peak area of the allele identified. The percentage contamination can be assessed by comparing the peak area of a contaminating peak to the peak area of a non-shared allele.

## 3.3 Results

DNA samples were extracted for 58 participants of the PK clinical trial with parental consent. Paired buccal and blood samples were obtained for 45 babies to compare the quality and quantity. Eleven neonates were included in the sub-study to determine whether DNA was contaminated by the donor's DNA following transfusion. This subgroup had been transfused allogenic blood product (s) within a week prior to DNA sampling.

### 3.3.1 DNA Quantity and Quality

A total of 51 DNA samples were obtained from scavenged blood and 30 from buccal cells to compare the yield and quality of DNA. The mean amount of DNA obtained from buccal cells was 3.4 µg compared with 42.95 µg from scavenged blood ($P<0.0001$). The purity of DNA ratios A260:A280 were satisfactory for all DNA, but were significantly higher in buccal cell derived DNA (2.10 cf 1.88, $P<0.0001$). Low values could indicate protein contamination. A260:A280 and A260:A230 are indicators of DNA purity with expected values in the range of 1.8 – 2.2. However, the A260:A230 was significantly lower in buccal cell derived DNA (0.23 cf 1.88, $P<0.0001$) which may indicate salt carry over from the DNA extraction procedure. Figure 3-1 shows the spread of results. This may affect the efficiency of future the PCR reactions.

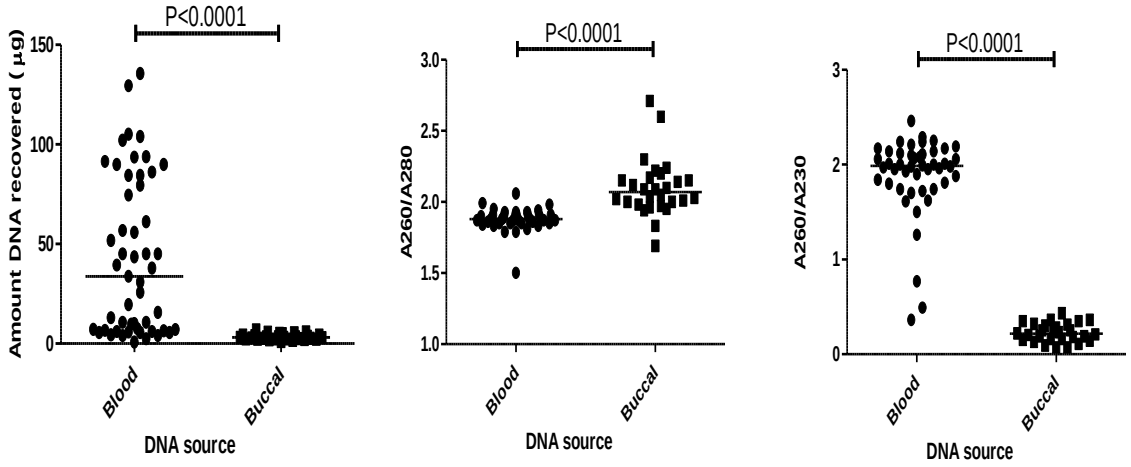



Figure 3-1 DNA Quantity and Quality of DNA from Buccal or Blood samples

Blood scavenged from clinical samples compared to a buccal scrape (n=45)

### 3.3.2 Contamination by donor blood following allogenic transfusion

The shortest time between the DNA sample being taken and the most recent administration of an allogenic blood transfusion was no greater than a maximum of four days. This sub-group were inpatients for between 11 to 132 days (median 55) and during this period they were administered up to 38 transfusions (median n= 18).

The transfusion service issue gamma irradiated products when neonates are diagnosed with suspected Di George syndrome or if administered an inter-uterine blood transfusion. Occasionally an irradiated product will be administered because it is readily available or when it is about to expire. Fresh frozen plasma and cryoprecipitate are routinely treated with methylene blue. In this group LW35 received 2 irradiated products on 19th and 25th January 2012 but this was after the DNA sample was collected. None of these products were treated with methylene blue.

Table 3-2 Blood products administered during the admission

| ID | Transfusions | | | | | | Total Days of Admission |
|---|---|---|---|---|---|---|---|
| | Prior to DNA Sample | | | | During the admission | | |
| | Packed Red Cells or SAGM | Platelets | Total blood products | Days before DNA | Total | Irradiated Products | |
| LW01 | 11 | 2 | 13 | 1 | 31 | No | 102 |
| LW02 | 5 | 4 | 9 | 1 | 20 | No | 85 |
| LW11 | 3 | 0 | 3 | 1 | 9 | No | 11 |
| LW12 | 1 | 0 | 1 | 1 | 4 | No | 26 |
| LW14 | 5 | 4 | 9 | 4 | 18 | No | 26 |
| LW16 | 3 | 0 | 3 | 1 | 14 | No | 132 |
| LW17 | 4 | 0 | 4 | 1 | 20 | No | 44 |
| LW20 | 6 | 0 | 6 | 1 | 6 | No | 115 |
| LW22 | 4 | 0 | 4 | 2 | 8 | No | 38 |
| LW33 | 1 | 1 | 2 | 1 | 38 | No | 55 |
| LW35 | 4 | 0 | 4 | 3 | 13 | Yes** | 85 |
| Mean | 4.3 | 1 | 5.2 | 1.5 | 16.4 | | 65.4 |
| Median | 4 | 0 | 4 | 1 | 18 | | 55 |
| Q1-Q3 | (3-5) | (0-2) | (3-9) | (1-2) | (8-20) | | (26-102) |
| SD | 2.72 | 1.6 | 3.64 | 1.03 | 10.55 | | 40.5 |

***FFP was not administered to the sub-group ** (2 units after DNA sample)*

### 3.3.3 Genotyping

Representative electropherograms from Powerplex analysis are shown in Figure 3-2 and 3.3. On detailed inspection of the electropherograms there were very low concentrations of alleles that could have arisen from donor contamination. Figure 3-3 illustrates a Y peak observed in female sample. The contaminating Y peak area = 223, non-shared allele peak area = 32133, estimated contamination = 223/32133 x 100 = 0.69%. Estimated contamination was less than 1% for all alleles observed. Sample LW14 showed the most potential contamination of all the samples, with additional peaks observed in the blood derived DNA for at least 4 of the 16 loci tested. It is possible that additional peaks were present but undetectable in other loci due to being “lost” in baseline noise. In this female patient a ‘Y’ allele was present therefore suggesting contamination from a male donor but the donor details are not available to confirm this.

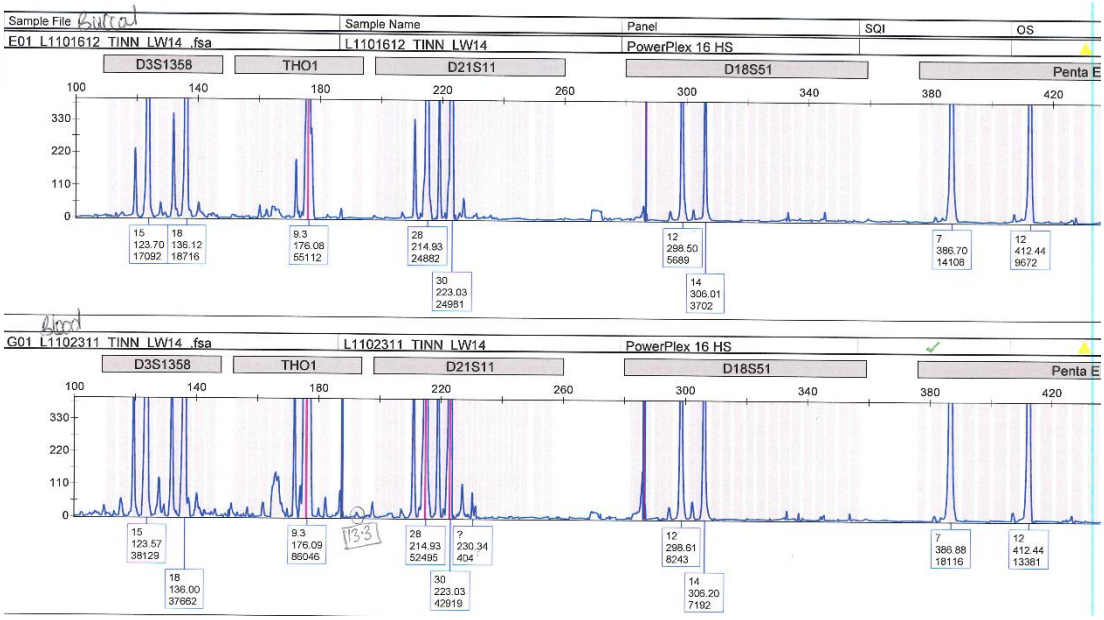



Figure 3-2 Electropherogram of a Buccal and Blood Samples (LW14)

Different coloured traces represent different fluorescent labels. Peaks are labelled according to molecular size, allele assigned to each locus and peak area. The genotyping of the buccal and blood derived DNA samples matched when using the standard analysis used in clinical practice.

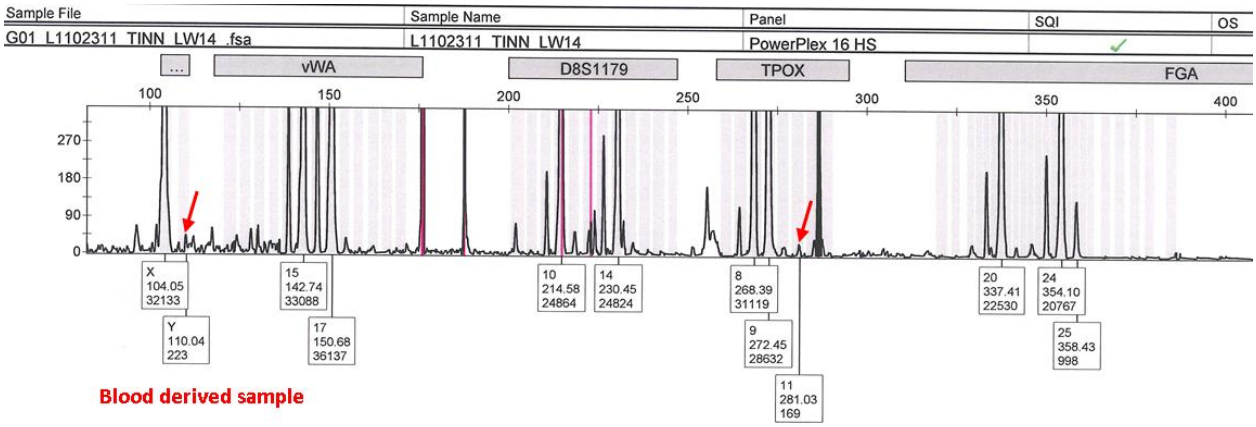



Figure 3-3 Blood Derived DNA Electropherogram (LW14)

### 3.4 Discussion

A substantially larger quantity and higher quality of DNA was obtained for pharmacogenomic analysis than was available from a buccal scrape. This study suggests that despite these babies being transfused frequently and immediately prior to DNA sampling, the genotyping of allelic polymorphisms in transfused babies did not affect genotyping data.

Scavenging blood from clinical samples that would otherwise be discarded is beneficial. As these babies were critically ill they require frequent clinical sampling, often leading to multiple blood transfusions due to their small circulating volume [79]. This sub-study therefore represents a particularly high risk group; these babies were frequently transfused with as many as thirteen blood transfusions prior to DNA sampling and up to 38 transfusions during their admission, (median n=18). Many babies (70%) had been transfused within 24 hours prior to DNA sampling for this comparison. Some studies suggest this can persist for years and be as much as 5% of circulating leucocytes [214, 215]. Prior to leukocyte depletion in 1998 Vervoordeldonk reported microchimerism persisted for several weeks in post transfusion patients [289]. More recently, Gong *et al.* [290] found similar results to these neonatal data; they reported no major effects on the genotype of common polymorphisms when DNA was collected within a day after transfusion.

The risk of donor contamination is reduced since leukocyte depletion of blood products however, the white cells are not totally eradicated and there is an argument that circulating leukocytes increase after 2-3 days [290, 291]. In theory this may result in a higher risk of DNA contamination after 2-3 days. This was not evident in this neonatal population. The median time between transfusion and DNA sampling was 1.5 days, however, 90% of these babies had additional transfusions prior to this

(median 4). Therefore the increase in leucocytes over time as a result of prior transfusions does not appear to have resulted in DNA contamination.

Even in this high risk group with recent multiple transfusions these data suggest that DNA testing following transfusion does not need to be delayed due to microchimerism. The frequency of clinical sampling provides an opportunity to scavenge discarded blood for DNA avoiding further blood loss and distress to babies when required for research. Genotyping of the DNA identified some additional peaks after zooming in but concentration of contamination was negligible these peaks were not frequent enough within the 16 allelic groups to suggest donor contamination. Donor contamination may have been present in a few samples but at such a low concentration that it would not have affected genotyping. The peaks were not big enough to constitute a high enough proportion of the source DNA to change genotyping results or other results from pharmacogenomic studies. These rare peaks may have been artifacts or stutters and would have required comparison with the donor's DNA to confirm this. These data have shown that irrespective of whether a low concentration of donor cells may be present this has not interfered with the genotype. A limitation of the study is that samples were assessed retrospectively therefore the donor's blood was not available for comparison. A prospective study would be required to validate this by comparing the true peaks of the donor blood against possible artefacts and relate to data such as gender of the donor.

In conclusion, DNA extracted from clinically required samples in critically ill neonates has provided a greater quantity and quality of DNA than buccal cells. Genotyping has shown that the DNA is unlikely to have been significantly contaminated by donor cells despite being transfused less than a week before DNA sampling.

# Chapter 4 Microbiological and Clinical Outcome

## 4.1 Overview

This Chapter describes a retrospective study of neonates with confirmed Gram-negative septicaemia over a six year period. Organisms were cultured and the MIC determined using an E Test. For a sub-group administered ciprofloxacin (5mg/kg 12 hourly) each neonate's clinical outcome was compared to the minimum inhibitory concentration (MIC) of ciprofloxacin required to inhibit growth of the Gram-negative isolate identified in their blood culture. The change in illness severity score before and after confirmation of Gram-negative bacteria in blood cultures was assessed.

The annual changes in MIC of Gram-negative organisms over this period were compared to assess MIC creep defined as a change in Ciprofloxacin MIC distributions over time so that strains with higher MICs become more common [292]. Due to concerns regarding resistance, data from surveillance surface swabs over a thirteen year period was summarised. This allowed a comparison of the incidence of bacteria resistant to gentamicin (the first line antibiotic) with ciprofloxacin.

The laboratory assessments of MIC and cultures, retrospective review of the clinical outcome and statistical analysis were completed by the research fellow.

### 4.1.1 Primary Aim and Objectives

**Aim:**

To describe the microbiological and clinical aspects of the use of ciprofloxacin on a Neonatal Intensive Care Unit using a retrospective cohort study.

**Objectives:**

- **Distribution of MIC:** To examine the distribution of the MIC of ciprofloxacin among Gram-negative organisms isolated in blood cultures from in-patients on a neonatal intensive care inpatients
- **Clinical Outcome:** to determine the clinical outcome for Gram-negative septicaemia in neonates treated with ciprofloxacin 5mg/kg 12 hourly according to the organism and the MIC
- **Illness Severity**: To assess the change in the severity of illness (NTISS score) when Gram-negative organisms in blood cultures are confirmed.
- **Sub-clinical resistance:** to assess whether frequent use of ciprofloxacin in Liverpool Women's NHS FT Neonatal Unit had an effect on MIC over time by comparing annual changes in MIC.
- **Surveillance:** To compare the incidence of organisms resistant to ciprofloxacin and gentamicin reported on surveillance swabs including throat and rectal.

## 4.2 Methods

The study was approved by the Trust and University Research and Development Departments and the National Research Ethics Service (10/H1002/79). Consent from families was not required for the retrospective analysis of data as samples were collected as part of routine clinical practice.

### 4.2.1 Distribution of the Minimum Inhibitory Concentration (MIC)

The MIC for ciprofloxacin of isolates retrieved from blood cultures were assessed using the EUCAST methodology [155]. The MIC was defined as the lowest concentration (mg/L) of antimicrobial that under defined *in vitro* conditions prevented the growth of bacteria within a set period of time [155]. Based on the clinical breakpoint set by EUCAST non-species specific isolates treated with ciprofloxacin were classed as susceptible ≤0.5mg/L, intermediate >0.5 to 1mg/L and resistant to ≥1mg/L [159].

Table 4-1 EUCAST non-species specific clinical breakpoints

| **Standard** | **E Test** | | **Disc Test** | | |
|---|---|---|---|---|---|
| | **MIC breakpoint (mg/L)** | | **Disc content (µg)** | **Zone diameter (mm)** | **Zone diameter (mm)** |
| **EUCAST** | **S ≤** | **R >** | | **S ≤** | **R >** |
| **Ciprofloxacin** | 0.5 | 1 | 5 | 22 | 19 |
| **Control *E Coli* ATCC 25922** | 0.008 | 0.004-0.015 | 5 | 35 | 30-40 |

S= Susceptible and R = Resistant

Gram-negative organisms isolated from blood cultures were routinely stored on beads in the clinical diagnostic laboratory at -80ºC between 2004 and 2010. For the purpose of this evaluation they were transferred temporarily to the study -80°C freezer in the clinical diagnostic laboratory (without defrosting in transit). As part of the clinical diagnosis Gram-negative organisms had been classified to both genus and species level using API strips: using biochemical and enzymatic reactions (http://www.biomerieux-diagnostics.com/apir-id-strip-range accessed August 2015). These data were retrieved from the patient data system

A total of 112 isolates from 103 patients were retrieved from storage and were cultured. Colombia agar plates were incubated for 18 to 20 hours in air at 37ºC. A suspension was prepared using colonies that formed after overnight growth by removing a single colony with a loop and emulsifying in 2 mL of sterile water. The EUCAST standard method requires the inoculum turbidity to be equivalent to 0.5 McFarlane standard solution and this was confirmed using a photometric turbidometer (Optek Germany). Further colonies or water were added to the inoculum to achieve the exact turbidity. Two Muller Hinton agar plates were then inoculated within 15 minutes of preparing the inoculums. A swab was used to inoculate the plates and achieve an even growth.

A ciprofloxacin E Test strip (AB bioMerieux Sweden) was applied to one plate using flamed forceps. The antimicrobial disc test (Oxoid Hampshire England) for both ciprofloxacin and nalidixic acid were added to the second plate. 112 plates had the E Test ciprofloxacin applied. The plates were then incubated within 15 minutes for 18-20 hours and inverted to prevent humidity contaminating the plate. The plates were assessed to ensure an acceptable inoculum density produced by a confluent lawn of growth such as that shown on the 3rd plate.

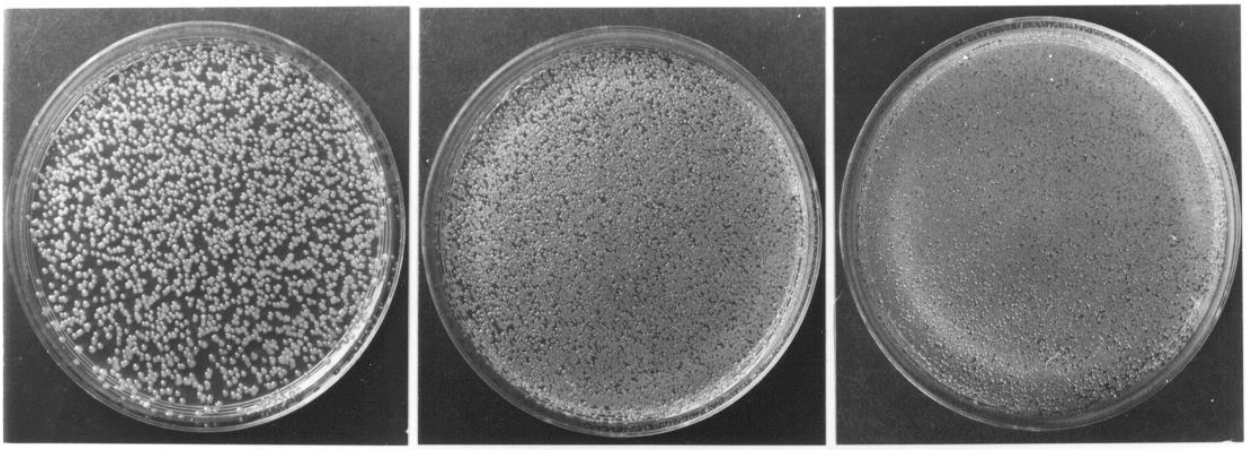


Figure 4-1 E Test confluent lawn of growth

A ciprofloxacin E Test strip (AB bioMerieux Sweden) was applied to one plate using flamed forceps. The antimicrobial disc test (Oxoid Hampshire England) for both ciprofloxacin and nalidixic acid were added to the second plate. 112 plates had the E Test ciprofloxacin applied.

Nalidixic acid is an early synthetic fluoroquinolone that has less activity than ciprofloxacin therefore is more sensitive to identifying resistant strains. For comparison 25 plates were inoculated and had a nalidixic acid E Test applied. In addition a further 112 plates had both a nalidixic acid and ciprofloxacin disc test to compare the ability of nalidixic acid to more readily detect resistance for ciprofloxacin.

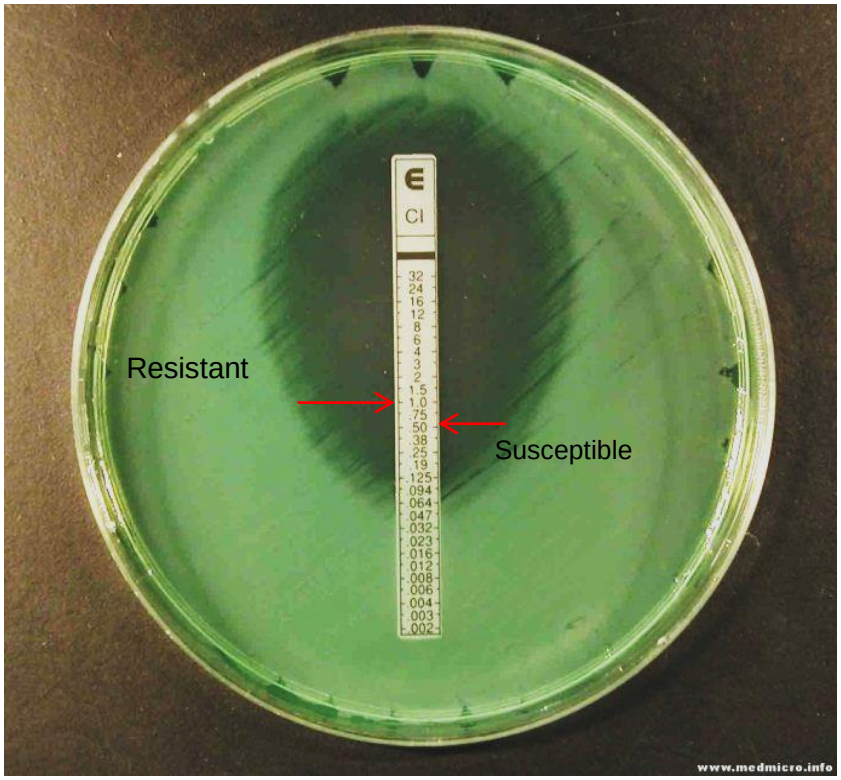



Figure 4-2 E Test for ciprofloxacin

The EUCAST MIC breakpoints for susceptibility and resistance are indicated by arrows on the E Test strip. This figure illustrates a susceptible strain of Pseudomonas aeruginosa with arrows indicating the clinical breakpoint.

For quality control, an *E. coli* inoculum was used with a known MIC. After incubation the zone edge was read at the point of complete inhibition as judged by the naked eye with the plate held about 30 cm from the eye. Discrete colonies growing within zones were sub-cultured to identify contamination with other organisms and then repeated if required. The MIC of each individual organism was categorised as susceptible, intermediate or resistant based on EUCAST breakpoints. The data was coded using a laboratory number, but not the patient's details, to ensure the investigator was blinded to the results.

### 4.2.2 Clinical Outcome Cohort

Clinical outcomes were assessed retrospectively. The isolates were retrieved and re-cultured for comparison with the MIC. Retrospective data for the short-term clinical outcome of neonates with Gram-negative infection was determined from the patient data system (BADGER) and the case notes.

#### 4.2.2.1 Inclusion criteria:

- A Gram-negative organism isolated from blood cultures whilst an in-patient on the Neonatal Unit at Liverpool Women's NHS FT between 2004 and 2010.
- Administration of ciprofloxacin 5mg/kg 12 hourly within 48 hours of a confirmed blood culture and continued for a minimum of five days.
- The isolate could be retrieved and re-cultured from storage at the Microbiology Laboratory for Liverpool Women's NHS FT.

The primary outcome of 'cure' was defined as survival and a C-reactive protein <10mg/L on the 10$^{th}$ day after confirmed Gram-negative infection. Neonates who had an elevated C-reactive protein or died within the 10$^{th}$ day following a confirmed Gram-negative blood culture were classed as treatment failure. Due to the high incidence of co-morbidity in this population if an elevated CRP or mortality occurred after the 10 day period this was not viewed as a direct temporal relationship with confirmed Gram-negative bacteraemia. There are limitations to outcome measures in neonates or critical illness (see section 1.5.1.1). This short term outcome was chosen as it is believed to be more sensitive to a direct link between the intervention and the outcome following administration of this antibiotic. Similarly, C-reactive protein (CRP) may have a temporal association with the intervention [293]. CRP was routinely monitored as a component of clinical practice and these concentrations were also available for retrospective analysis. Blood cultures were taken as part of the standard screening protocol for suspected sepsis. Isolates are routinely stored by the microbiology laboratory.

Retrospective data for the short-term clinical outcome of neonates with Gram-negative septicaemia was determined from the patient data system (BADGER) and case notes. Further demographic data including gender, postmenstrual age at birth, post menstrual age at the time of Gram-negative infection, and illness severity were recorded.

### 4.2.3 Severity of Illness Score

A retrospective analysis of the severity of illness of all babies with confirmed Gram-negative organisms in blood cultures was undertaken. In total 103 medical records were reviewed to evaluate the impact of the severity of illness on clinical outcome. Each baby was scored two days prior to confirmed Gram-negative septicaemia and on the day of confirmed septicaemia using the validated Neonatal Therapeutic Intervention Scoring System (NTISS) [294]. This is a detailed score of 8 organ systems or therapies and a total of 66 values per baby that may be scored between 1- 4 depending on the severity of illness including seven domains:

- Respiratory
- Cardiovascular
- Drug Therapy
- Monitoring
- Metabolic/Nutrition
- Transfusion
- Procedural Vascular access

This validated score was selected as it provides continuous values relating to the clinical management of the patient rather than binary outcome data. Source data was collected from the medical notes, patient data system, drug prescriptions, blood prescriptions and administration records.

### 4.2.4 Change in MIC over time –Sub-clinical resistance

The Minimum Inhibitory Concentration (MIC) of Gram-negative organisms from blood cultures was measured to determine the annual changes in MIC over time over a period of six years.

### 4.2.5 Surveillance: relative resistance of gentamicin v ciprofloxacin

A retrospective study of blood cultures, surveillance throat and rectal (surface) swabs was performed to quantify the number of organisms resistant to ciprofloxacin compared to gentamicin over a 13-year period.

#### 4.2.5.1 Inclusion criteria:

- Neonates admitted to the Neonatal Unit 1997 - 2010

The microbiology database for a tertiary neonatal unit was searched for Gram-negative isolates from surface throat and rectal swabs between 1997 and 2010. All Gram-negative organisms from surface swabs were routinely tested for gentamicin and ciprofloxacin sensitivity. Clinically and epidemiologically relevant organisms were reported throughout this period this included Gram-negative bacilli (Enterobacteriaceae & *Pseudomonas aeruginosa*) and Gram-positive cocci (*Staphylococcus aureus, Group B & Group A streptococci, Enterococci, MRSA*).

### 4.2.6 Statistical Analysis

All statistical procedures were performed in SPSS software (version 18 SPSS Inc. Chicago IL USA) and Minitab statistical software (version 16 Inc. Coventry UK).

The distribution of MIC to ciprofloxacin among different groups of Gram-negative organisms the median and IQR ciprofloxacin MIC were calculated and plotted. Clinical Outcome (clinical cure or treatment failure) and its relationship to MIC included a comparison of babies who did and did not meet the primary outcome of clinical cure. These were presented as medians and interquartile ranges or percentage frequencies. Univariate statistical analyses were performed using the Mann-Whitney U test for continuous data

except for categorical frequency data, which were analysed using a chi-squared test or Fisher's exact test. (Insufficient events were available for explanatory variables for linear regression). The optimal MIC values to predict cure were computed to produce an area under the Receiver Operating Characteristic (ROC) curve including sensitivity and specificity values (null hypothesis AUC 0.5). The optimal MIC value from the ROC analysis was used to define low and high MIC groups within the susceptible range for comparison with short-term outcome. Where appropriate differences were considered significant at the $p<0.05$ concentration, and results are presented with 95% confidence intervals. Positive predictive value was determined by a two by two table using vassarstats.net/clin2.html.

The NTISS score on the day of confirmed septicaemia and two days earlier were compared using median and IQR to determine the change in illness severity following confirmed Gram-negative infection. NTISS was correlated for comparison with gestational age at birth and at Gram-negative blood culture. The change in NTISS over the two days was compared. Clinical outcome of babies with Gram-negative infection was then compared using regression analysis to determine whether the outcome was associated with the severity of illness prior to Gram-negative infection.

The combined MIC for all organisms collected during each year of the study were compared and presented as the median and 95% CI to determine whether there was a visual increase in MIC over time. The percentage of isolates resistant to ciprofloxacin was compared to those resistant to gentamicin. Non-duplicate surveillance results were tabulated.

### 4.3 Results

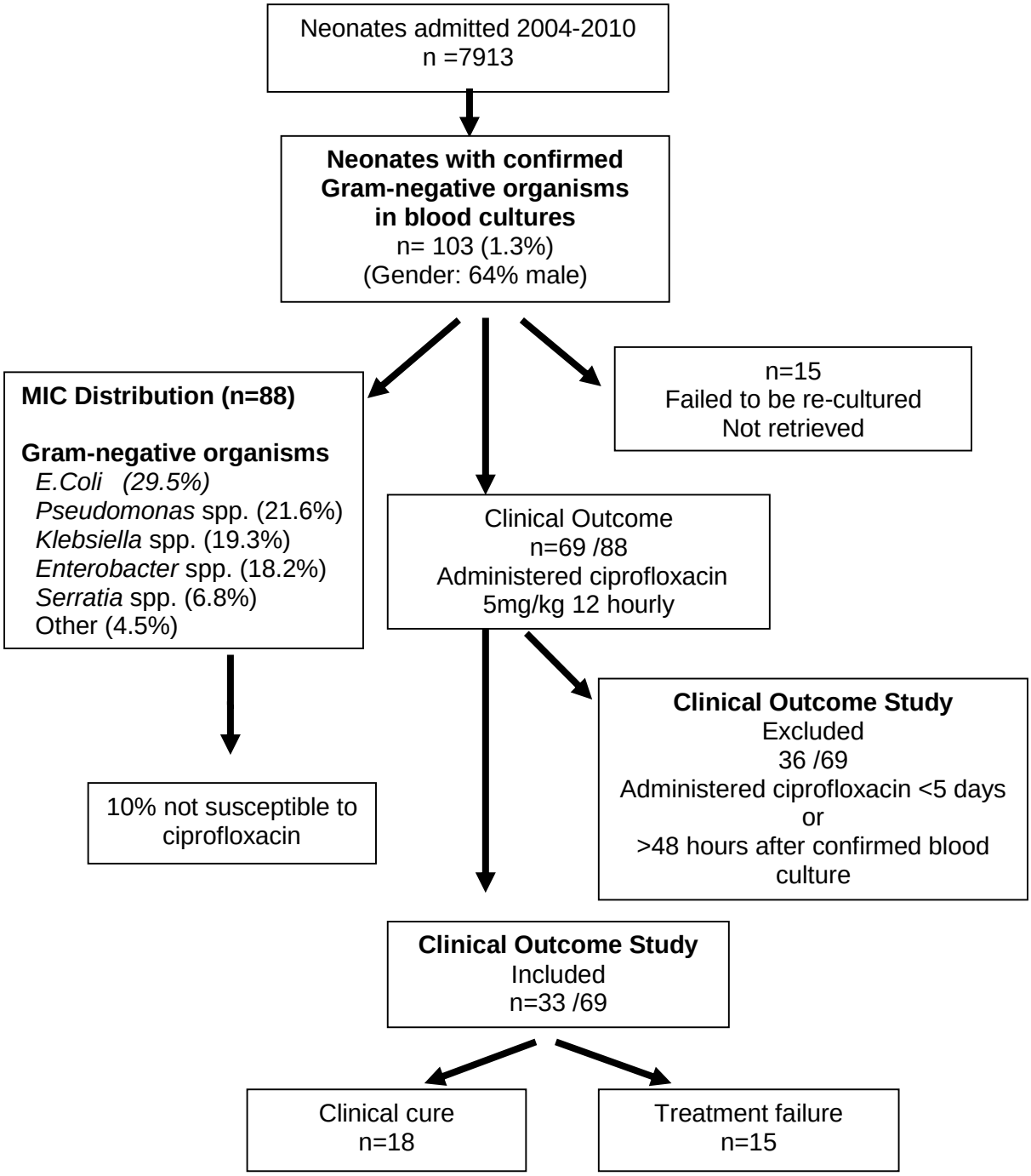



Figure 4-3 MIC Ciprofloxacin Distribution and Clinical Outcome of Gram–negative bacteraemia

The MIC ciprofloxacin distribution and Clinical outcome of neonates administered ciprofloxacin with confirmed Gram–negative bacteraemia between 2004 and 2010 (n=103)

### 4.3.1 MIC Ciprofloxacin Distribution: Gram-negative Blood Cultures

The incidence of Gram-negative organisms in blood cultures between 2004 -2010 was 1.3%. Eighty-eight of 103 original blood culture isolates were successfully re-cultured to assess the organism's MIC to ciprofloxacin (15 either failed to be re-cultured or retrieved). The most common organisms associated included *Escherechia*, *Pseudomonas* spp., *Klebsiella* spp. and *Enterobacter* spp. Table 4-2. The median and inter-quartile range (IQR) ciprofloxacin MIC for each of the main organism groups were below the susceptibility breakpoint 0.5 mg/L Figure 4-4. Amongst all organisms, *Pseudomonas* spp. and *Serratia* spp. had a higher median MIC. The main types of organisms (n=88) are shown in Table 4-2. Nine (10%) organisms were classed as not susceptible to ciprofloxacin >0.5mg/l; 6 (6.8%) of these isolates were classed as frankly resistant MIC ≥1mg/l and 3 (3.4%) organisms were classed 'I' intermediate >0.5 <1mg/L.

Table 4-2 Gram-negative organisms in blood cultures 2004 – 2010

| **Organisms (n=88)** | | | **MIC non-susceptible ≥0.5mg/L** | |
|---|---|---|---|---|
| | N = | (%) | N = | **(%)** |
| *E.coli* | 26 | (29.5) | 2 | (2.2) |
| *Pseudomonas* spp. | 19 | (21.6) | 2 | (2.2) |
| *Klebsiella* spp. | 17 | (19.3) | 2 | (2.2) |
| *Enterobacter* spp. | 16 | (18.2) | 0 | (0) |
| *Serratia* spp. | 6 | (6.8) | 2 | (2.2) |
| Other | 4 | (4.5) | 1 | (1.1) |
| **Total** | 88 | **(100)** | 9 * | **(9.9)*** |

*6 intermediate 0.5 – 1mg/l and 3 resistant ≥ 1mg/l

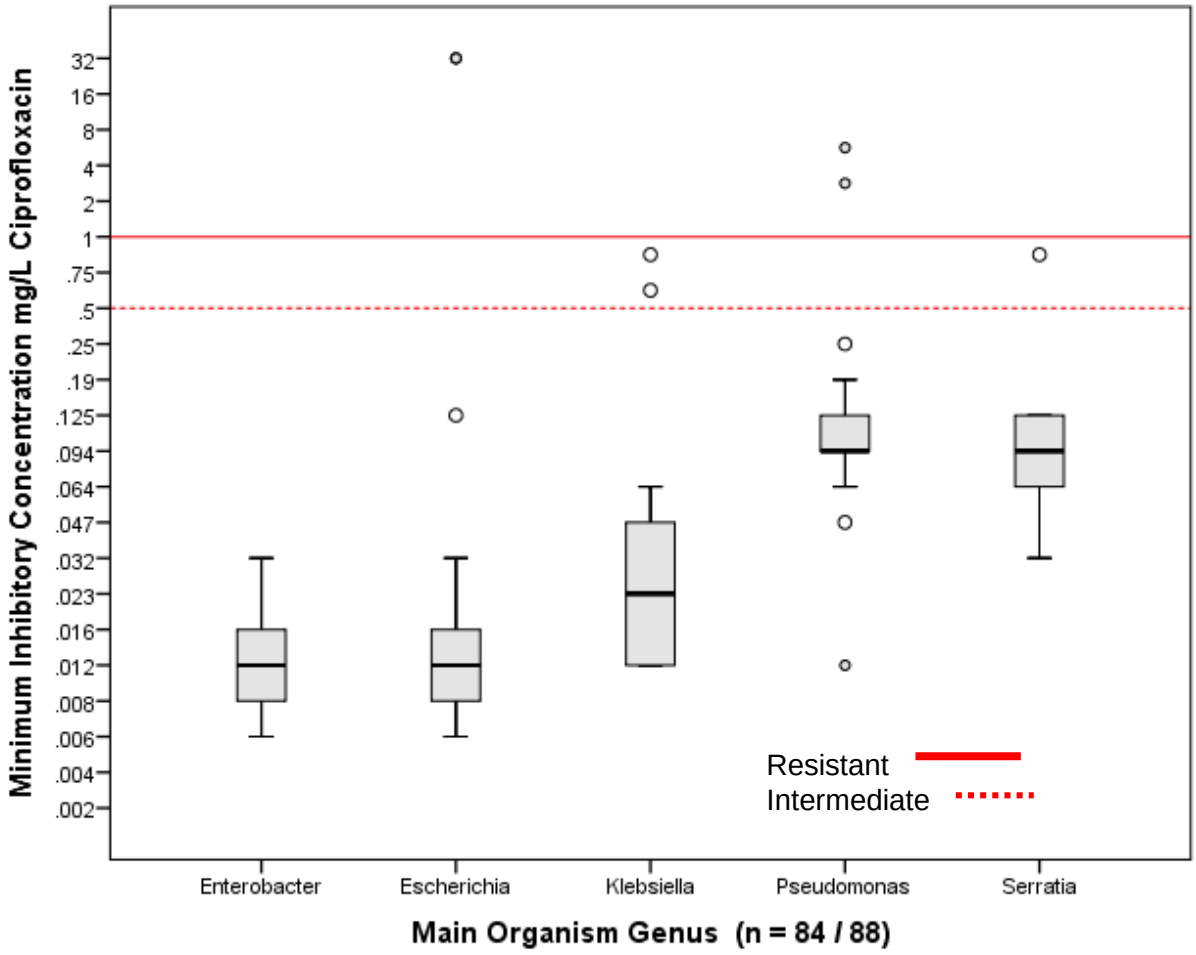



Figure 4-4 Ciprofloxacin MIC (mg/L) for Gram-negative organisms in blood cultures

Blood cultures were collected from neonates during 2004 -2010 (n=84) these data exclude isolated incidence of 4 organisms (*Acinetobacter , Citrobacter, Morganella and Raoultella).*

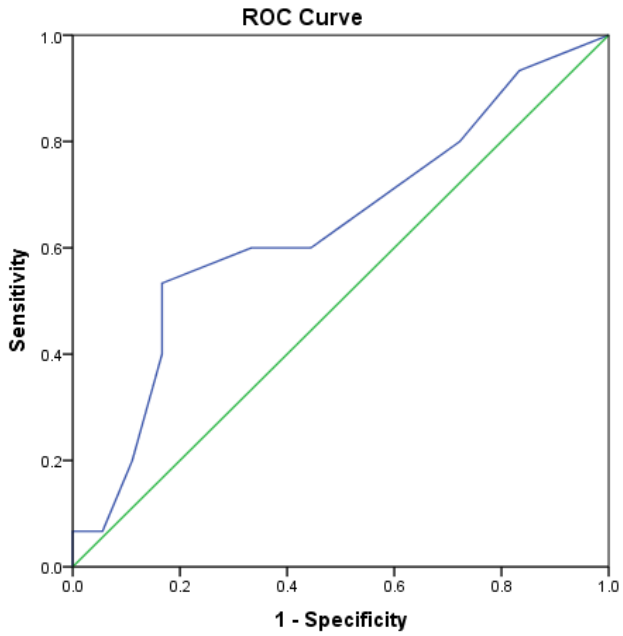
#### 4.3.1.1 Clinical Outcome of Gram-negative Bacteraemia v MIC Ciprofloxacin

A sub-group of neonates were treated with ciprofloxacin 5mg/kg 12 hourly. The clinical outcome was compared and the optimal sensitivity and specificity for cure assessed by the ROC analysis that defined a low and high MIC group. Figure 4-5 and Figure 4-6. This was further assessed by the positive predictive value.

Table 4-3 Clinical Outcome: Gram-negative Bacteraemia v MIC Ciprofloxacin (n=33)

| Variable | N= | Clinical Cure (n=18) | Treatment Failure (n=15) | Difference (95% CI) | *p* |
|---|---|---|---|---|---|
| **Ciprofloxacin MIC** | 33 | | | | |
| Median | | 0.012 | 0.047 | -0.009 | 0.17 |
| IQR | | 0.008-0.023 | 0.012 - 0.094 | (-0.078,0.0019) | |
| Range | | 0.006-0.190 | 0.0060 - 0.75* | | |
| **High and Low MIC groups (indicated by ROC analysis)** | | | | | |
| Total susceptible MIC Low | 33 | 18 (56%) | 15 (47%) | | 0.061 |
| MIC ≤0.035 | 22 | 15 (68%) | 7 (32%) | | |
| High MIC >0.035–0.5 | 11 | 3 (27%) | 8 (73%) | | |
| **Positive Predictive Value** | | | | 0.68 | |
| **Area under the curve** | | | | 0.612 (0.407, 0.817) | |
| **GNO (n=33)** | | | | | |
| *E. Coli* | 9 | 4 (44%) | 5 (56%) | | |
| *Pseudomonas* spp. | 6 | 2 (33%) | 4 (67%) | | |
| *Klebsiella* spp. | 7 | 3 (43%) | 4 (57%) | | |
| *Enterobacter* spp. | 9 | 8 (89%) | 1 (11%) | | |
| *Serratia* spp. | 2 | 1 (50%) | 1 (50%) | | |
| **Total** | | **18 (55%)** | **15 (45%)** | | |
| **PMA *Week (w) day(d)*** | | | | | |
| **PMA at b**irth (Median) | | 25 *w* 2**8** *w* 4 *d* | 25 *w* 5 *d* 29 *w* 4 *d* | 4 (-5,12) *d* 10 (-7,39) *d* | 0.33 0.26 |
| PMA at GN Sepsis (Median) | | | | | |
| **Gender** | | | | | |
| **Male** | | 13 | 9 | | |
| **Female** | | 7 | 4 | - 4% (-36.9%, 4.8%) | 0.8 |
| **Illness Severity NTISS (**Median IQR) | | 12.5 (8-17) | 16 (14-21) | - 4 (08.9,-0.002) | 0.04 |

**The MIC of one organism was 0.75 and had not been classed as resistant by the disc test.*




| Positive if > or equal to[a] | Sensitivity | 1- Specificity |
|---|---|---|
| .000000 | 1.000 | 1.000 |
| .007000 | .933 | .833 |
| .010000 | .800 | .722 |
| .014000 | .600 | .444 |
| .019500 | .600 | .333 |
| **.035000** | **.533** | **.167** |
| .055500 | .467 | .167 |
| .079000 | .400 | .167 |
| .157500 | .067 | .056 |
| .470000 | .067 | .000 |
| 1.000000 | .000 | .000 |

**Area Under the Curve**

Test Result Variable(s): Minimum Inhibitory Concentration of Ciprofloxacin mg/L

| Area | Std. Error [a] | Asymptotic Sig.[b] | Asymptotic 95% Confidence Interval | |
|---|---|---|---|---|
| | | | Lower Bound | Upper Bound |
| .612 | .105 | .285 | .407 | .817 |

The test result variable(s): has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption b. Null hypothesis: true area = 0.5

Figure 4-5 Receiver Operator Curve - optimal MIC mg/L for cure (n=33)

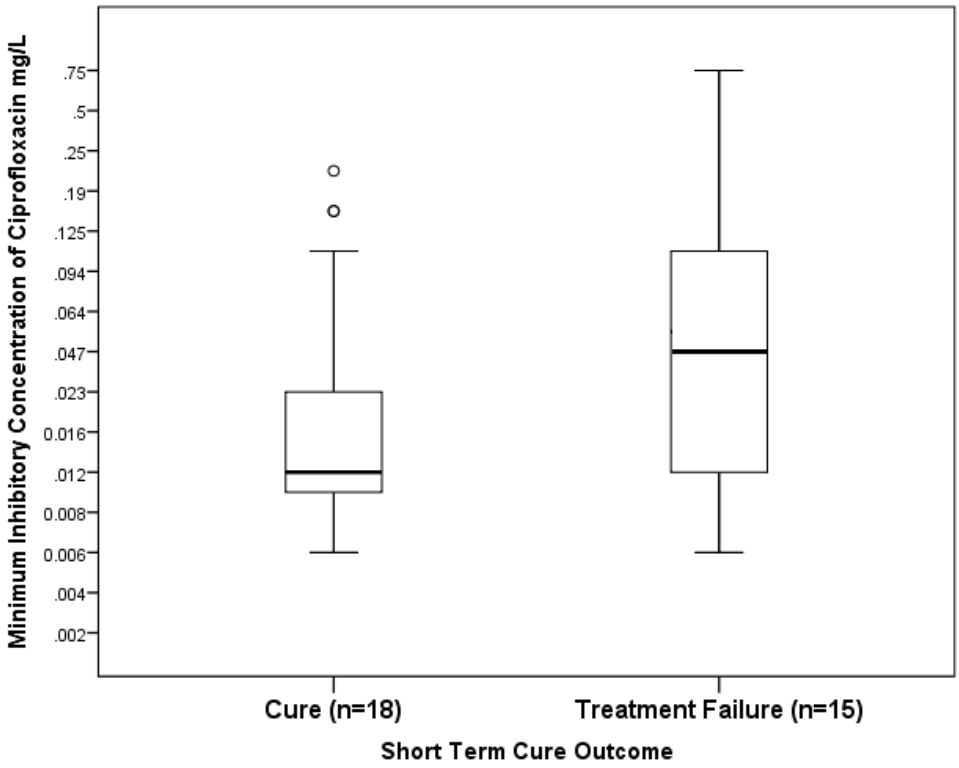



Figure 4-6 Clinical Outcome of neonates treated with Ciprofloxacin v MIC (Mg/L)

The clinical outcome of neonates treated with ciprofloxacin for Gram-negative bacteraemia v MIC Ciprofloxacin (n=33) Ciprofloxacin MIC median for cure was 0.012 (IQR 0.008 -0.023) compared to treatment failure 0.047 (IQR 0.012 to 0.094).

Table 4-4 Mortality and morbidity for neonates with Gram-negative organisms (GNO) in blood cultures 2004 – 2010 (n=103)

| **Mortality and Co-morbidity** | N= | % |
|---|---|---|
| Mortality | 45 | (44) |
| within 10 days of GNO | 27 | (26) |
| within 28 days of GNO | 36 | (35) |
| after 28 days | 9 | (9) |
| GNO associated with mortality <10 days | | |
| *E.coli* | 9 | (33) |
| *Pseudomonas* spp. | 7 | (25) |
| *Klebsiella* spp. | 5 | (18) |
| *Enterobacter* spp. | 3 | (12) |
| *Other* | 3 | (12) |
| Co-morbidity (main) | | |
| Necrotising enterocolitis | 28 | (28) |
| Intraventricular haemorrhage | 51 | (51) |

Mortality for neonates with confirmed Gram-negative septicaemia was 44% prior to discharge or 26% within 10 days of confirmed Gram-negative septicaemia. The main organisms associated with mortality within 10 days included *E.coli*, *Pseudomonas* spp., *Klebsiella* spp. and *Enterobacter* spp. MIC over time – Sub-clinical Creep Resistance

MIC data was plotted to evaluate whether there was an annual increase in MIC within the susceptible range over time. There were 9/88 resistant organisms. Among the 79 susceptible isolates with MIC <0.5 mg/L (susceptible to ciprofloxacin) a change in MIC was not visibly associated with the year of isolation (Figure 4-7).

Table 4-5 Ciprofloxacin Resistant Organisms in Blood Cultures

| **Year** | **Intermediate** | **Resistant** | **Total n=9** |
|---|---|---|---|
| 2004 | | | 0 |
| 2005 | | *Acinetobacter* spp. | 1 |
| 2006 | | | 0 |
| 2007 | *Klebsiella* spp. (x2) | *Pseudomonas* spp. | 3 |
| 2008 | | *Pseudomonas* spp. | 1 |
| 2009 | | *E.Coli* | 1 |
| 2010 | *Serratia* | *E.Coli* (x2) | 3 |

This table includes resistant and Intermediate susceptible Organisms from blood cultures per year 2004 - 2010 (n=9 /88)

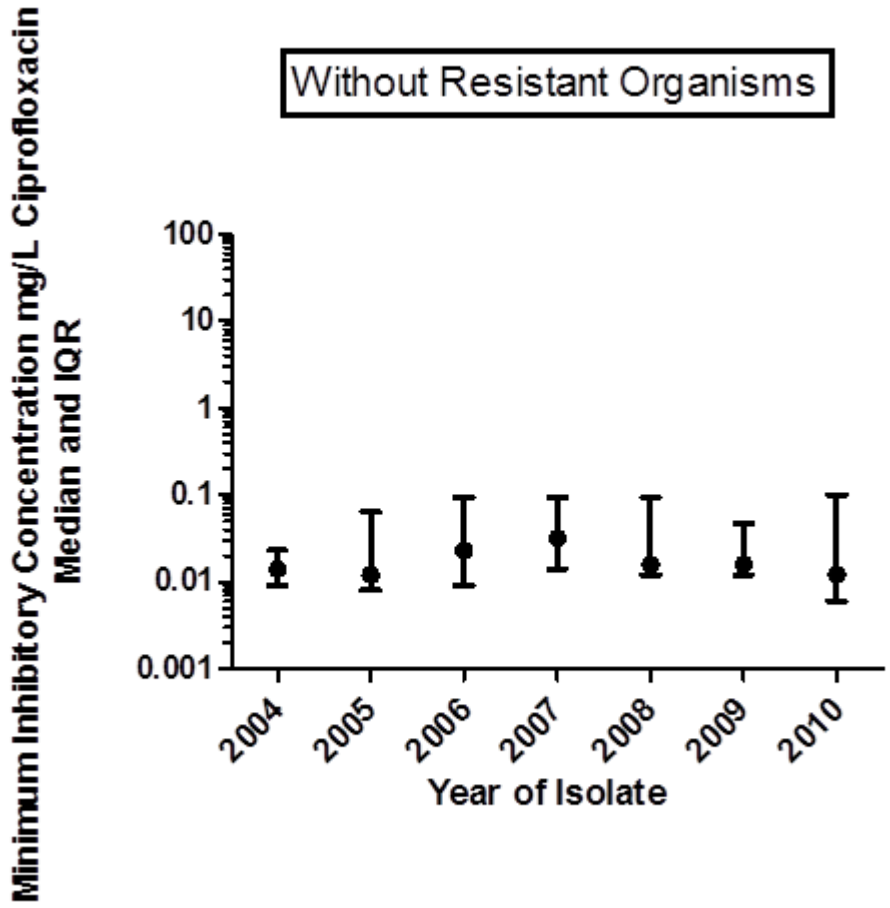



Figure 4-7 MIC mg/L (log 10) of ciprofloxacin for susceptible organisms (median and IQR) from blood cultures in neonates (n=79)

### 4.3.2 Surveillance: relative resistance to ciprofloxacin and gentamicin

#### 4.3.2.1 Surveillance surface swabs (throat and rectal)

Epidemiologically significant organisms on surface surveillance swabs (throat and rectal) were reported during 1997 – 2010) (Table 4-6). This resulted in 42,507 reports from routinely collected throat and rectal surface swab monitored on admission then weekly for 15,951 neonates. Therefore, these data provide the proportion of resistant Gram-negative organisms per annum for both gentamicin and ciprofloxacin. Gentamicin is the first line treatment for suspected Gram-negative sepsis whereas ciprofloxacin is generally the second line treatment. 209 babies (1.31%) of all admissions had one or more surveillance swabs with a Gram-negative organism not susceptible to gentamicin and 151 (0.95%) to

ciprofloxacin; these included 69 (0.43%) isolates that were not susceptible to both gentamicin and ciprofloxacin.

The most common resistant organisms included *E.coli, Acinetobacter* spp*. and Pseudomonas* spp*.* These data exclude *S. maltophilia* from further analysis as it is less susceptible to both gentamicin and ciprofloxacin therefore would not have been selected in clinical practice.

Table 4-6 Surveillance of surface swabs for neonates 1997 to 2010

| | N= | % |
|---|---|---|
| **Neonatal admissions** | 15 951 | |
| Average per annum | 1 227 | |
| **Surveillance with clinically relevant growth:** | | |
| **Swabs** | 42 507 | |
| **Neonates** (% of admissions) | 6 114 | (38) |
| Average swabs screened per neonate | 6.9 | |
| Blood culture organisms identified on surface swabs | | (40) |
| **Neonates Gram-negative organisms*** | | |
| Surface swabs | 5 059 | |
| **Epidemiologically relevant swabs**** | | (82 |
| Proportion of admissions | | (31) |
| Sensitivity tested for gentamicin | 5 059 | (100) |
| Sensitivity tested for ciprofloxacin | 3 944 | (78) |
| **Neonates with non-duplicate resistant Gram-negative organisms** | | **% of admissions** |
| Gentamicin Resistant | 195 | (1.22) |
| Intermediate resistant | 14 | (0.09) |
| **Total** | 209 | (1.31) |
| Ciprofloxacin* Resistant organism | 123 | (0.81) |
| Intermediate resistant | 28 | (0.18) |
| **Total** | 151 | (0.99) |
| Both gentamicin and ciprofloxacin (included in above) | | |
| Resistant | 54 | (0.34) |
| Intermediate | 15 | (0.09) |
| Total | 69 | (0.43) |

* Non-duplicate **Epidemiologically relevant included Gram-negative bacilli (*Enterobacteriaceae* & *Pseudomonas aeruginosa*) and Gram-positive cocci (*Staphylococcus aureus*, Group B & Group A *streptococc*i, *Enterococci*, *MRSA*).

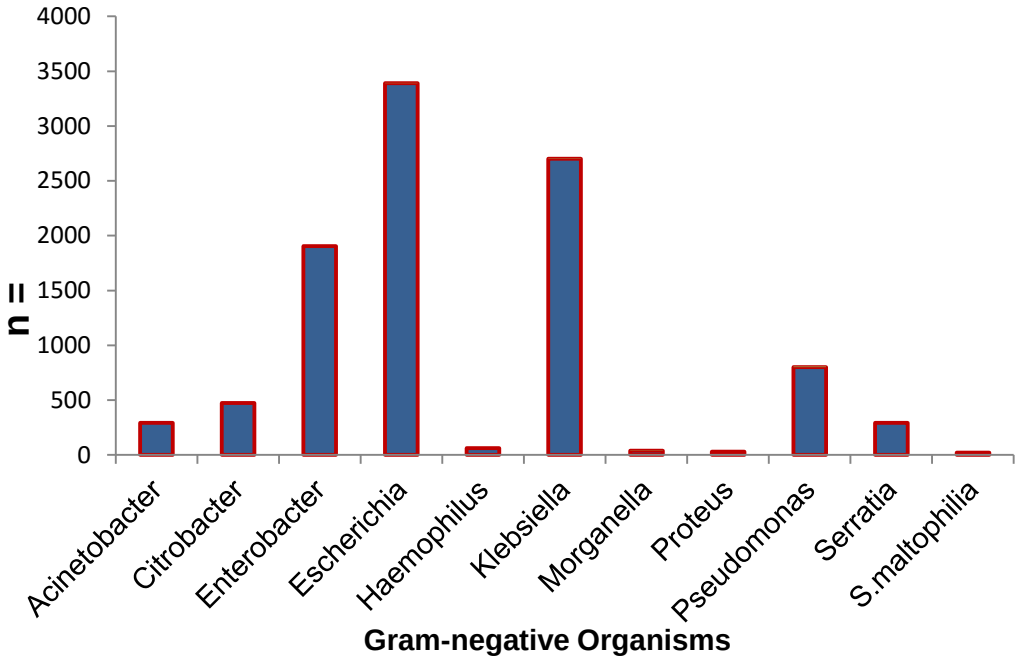



Figure 4-8 Gram-negative organisms on surface swabs

Gram-negative organisms (non-duplicate) epidemiologically relevant and identified on routine surveillance surface swabs between 1997 – 2010 (n=10,007)

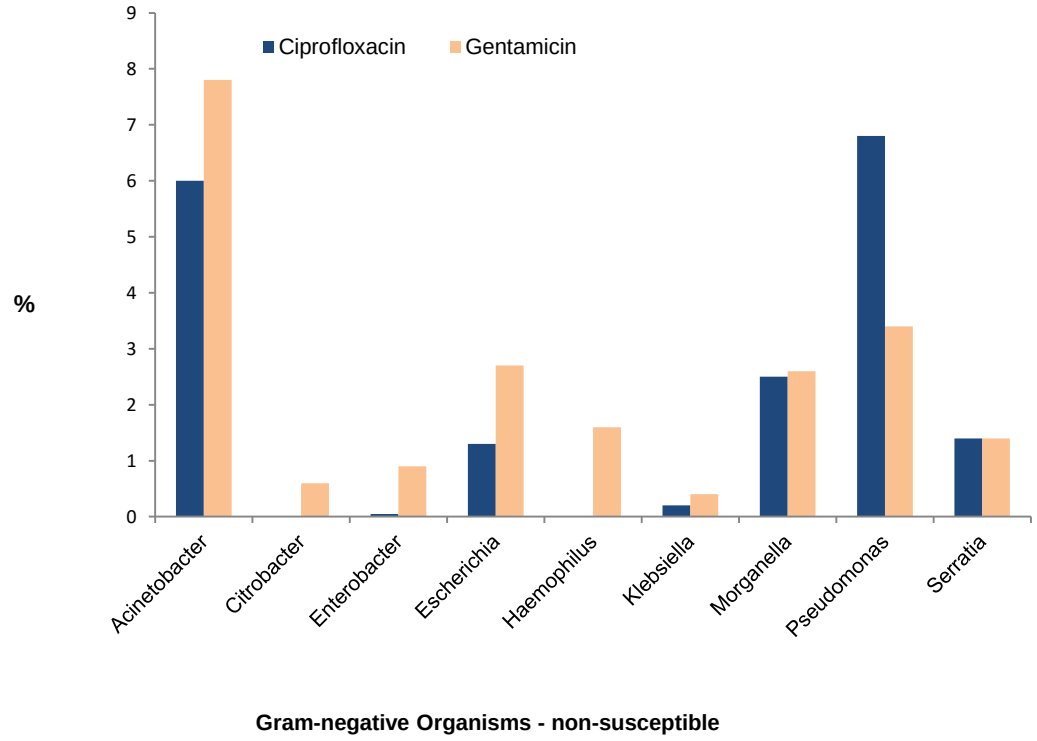



Figure 4-9 Non-susceptible Gram-negative Organisms

Illustrates the Gram–negative organisms (non-duplicate) not susceptible to ciprofloxacin (n=151) and gentamicin (n=209) between 1997 – 2010

### 4.3.3 Severity of Illness Score pre and post Gram-negative Bacteraemia

The Neonatal Therapeutic Intervention Scoring System (NTISS) illness severity score monitored the change in illness severity with confirmed Gram-negative organism. This was available for 79 neonates with Gram-negative isolates from blood cultures between 2004 and 2010 (*E. coli* 24, *Pseudomonas* spp. 16, *Enterobacter* spp.16, *Klebsiella* spp. 13, others 10). Two days prior to the isolation of Gram-negative bacteria NTISS had median 14, IQR 11 - 18, max 28 on the day a Gram-negative isolate was identified median 20, IQR 14 - 25, max 34.1 (n=70). NTISS was negatively correlated to gestational age at birth (rs = -0.344, p< 0.005) but was not associated with postnatal / postmenstrual age at culture, sex or the nature of the bacteria.

The NTISS changed over the two days by a median 5 (IQR 1 – 8) max 23 and was inversely correlated to NTISS 2 days before isolation of Gram-negative bacteria (-0.365, p<0.01). The higher the NTISS was two days before the isolation of GN bacteria the lower was the change in NTISS when infection started. Five neonates had lower NTISS scores when positive blood cultures were recognised compared to two days before. Each of these improvements was less than four NTISS units. Figure 4.10

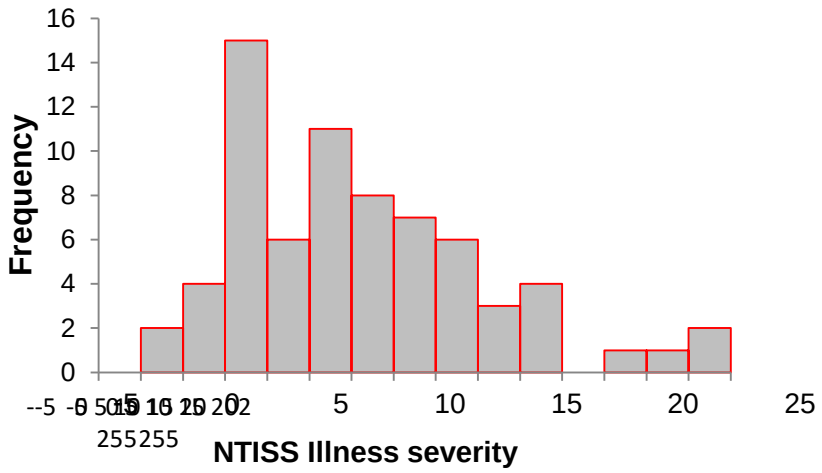



Figure 4-10 Illness severity score pre and post Gram-negative blood cultures

The Illness severity score NTISS was determined two days prior to and on the day of confirmed Gram-negative blood culture to assess the of the score to identify Gram – negative bacteraemia and the change in illness severity

## 4.4 Discussion

This chapter examined the clinical features and outcomes of neonates with Gram-negative bacteria in their blood cultures relative to the MIC ciprofloxacin and the incidence of Gram-negative organisms identified in surveillance data. This population were born extremely premature (96%) <31 weeks PMA and a high proportion (75%) were ≤31 when Gram-negative bacteraemia was confirmed. Mortality increased considerably to 44% when Gram-negative infection was confirmed in blood cultures compared with mortality rates in this unit for babies <28 weeks gestation at 20% and between 28-34 weeks gestation is 8%. Similar increases in mortality have been reported for late onset sepsis, Stoll *et al.* found mortality of VLBW neonates increased from 7% to 18% but when associated with Gram-negative organisms this increased considerably to 36% [18]. The extreme prematurity and complex co-morbidity confound the clinical outcome assessment of bacteraemia in this population. Many were severely ill as they presented with grade III and IV intra-ventricular haemorrhage (51%) and/or necrotising enterocolitis (28%) (Table 4-4).

### 4.4.1 MIC Ciprofloxacin Distribution: Gram-negative Bacteraemia

Gram-negative isolates in blood cultures are relatively uncommon. This neonatal unit has approximately >1200 admissions per annum, yet over a six year period 103 cases (1.3%) were reported (Figure 4-3). Most isolates were retrieved and successfully re-cultured (89%). The majority of organisms (90%) were classed as susceptible by EUCAST Figure 4-4. The least susceptible species were *Pseudomonas* spp. and *Serratia* spp. Figure 4-4. *Enterobacter* spp. had the lowest MIC and no resistant organisms were identified. The most common organisms were *E.Coli* (29.5%) followed by *Pseudomonas* spp. (21.6%) then *Klebsiella* spp. (19.3%) consistent with other studies [11, 18, 25, 26, 39, 40] (Figure 1-3). A UK survey found the most common bacteria causing late-onset sepsis were Gram-positive CoNS (45%), followed by *Staphylococcus aureus* (13%), then Gram-negative bacteria including *Enterobacteriaceae* (9%), *E. coli* (7%) and *Group B Streptococcal* (7%) [71].

Over a seven year period 10% of Gram-negative bacteraemia were resistant Table 4-5. MIC creep or sub-clinical resistance would illustrate a trend for increasing MIC within the susceptible range. This maybe present but was not evident from these data due to the low number of confirmed isolates in blood cultures over the time period of seven year period (n=79) Figure 4-7. There were less than 12 susceptible organisms per annum and there were different species within this small number that naturally have variation in MIC. As a result the median MIC fluctuated over this period but a visible trend was not evident Figure 4-7. Another limitation for assessing MIC creep is the possibility that there is cross infection within a neonatal unit that may result in a bias towards that organism's MIC which has greater influence on data when the numbers are so small. To observe organism specific MIC creep would be optimal but more challenging as even less isolates are available from bacteraemia. An option may be to a prospective study to determine MIC creep from surveillance isolates.

### 4.4.2 Clinical outcome v MIC: Gram-negative Bacteraemia

A subgroup of neonates were administered ciprofloxacin 69 (78%), of whom 33 (56%) met the eligibility criteria for assessment of their clinical outcome (Table 4-3). The MIC of the organism found in the neonate's blood culture was compared to the same neonate's clinical outcome. Relatively higher MICs were associated with a greater risk of treatment failure even when within EUCAST's susceptible range but was not statistically significant (p= 0.17) (Figure 4-6). ROC analysis Figure 4-5 indicated the optimal specificity and sensitivity was ≤0.035 mg/L (AUC 0.641 CI 0.445 – 0.836). Although this was non–predictive the optimal point defined two groups to compare the outcome; a low MIC group as ≤0.035 mg/L and high group >0.035mg/L. In the low MIC group ≤0.035, 68% neonates were cured compared to only 27% in the high MIC group >0.035 to 0.5mg/L (p=0.61). The risk of treatment failure for the high MIC group was much higher (RR 2.5 95% CI 0.9139 - 6.8391 p=0.074) with a positive predictive value of 79%. Falagas *et al.* found increased mortality when the Gram-negative organism had a higher MICs within the susceptible

range (RR, 2.39; 95% CI, 1.19 to 4.8). The authors concluded that there is a need to reconsider the treatment of organisms with relatively high MICs even when in the susceptible clinical breakpoint range [163]. In respect of fluoroquinolones, Labreche *et al.* reported several studies with declining susceptibility and the need to review of clinical breakpoints for Gram-negative infection in particular for blood stream infections [67].

This is clinically significant as the microbiology laboratory classed the majority of blood cultures (90%) as susceptible to ciprofloxacin over this six year period Figure 4-4. A considerable proportion 43% of these bacteraemia had an MIC higher than 0.035mg/L. Ciprofloxacin was a treatment option based on EUCAST's clinical breakpoints and these neonates may have been under treated increasing the risk of treatment failure by 2.5 (95% CI 0.9139 - 6.8391 p=0.074). Fortunately not all of the babies with confirmed Gram-negative sepsis were treated with ciprofloxacin (n=33/103) as at the time of this retrospective study the dose was lower (10mg/kg/day) than the current recommended dose 20mg/kg/day). At present the optimal ciprofloxacin regimens for neonates and each sub-age group remains unknown. These outcome data are limited but support the view that there is a need to review clinical breakpoints within the susceptible range. Also, to extend breakpoints specific to the regimen and outcomes for sub-age groups of children.

These retrospective data suggest a higher daily dose was required than the 10mg/kg/day administered during this period or a lower breakpoint. This is consistent with the higher daily dose was proposed as a result of PK data in children > 3months age by Lipman *et al.* They found that a paediatric dose of 20mg/kg/day produced a lower $AUC_{24}$ of 30-40 than their study of adult [115]. This AUC would only achieve the minimum $AUC_{24}$/MIC ratio of >125 for organisms with MIC >0.24mg/L ($AUC_{24}$/MIC 30/0.24 =125) and led to a recommendation to increase the regimen to 30 mg/kg/day for more severe infections [115]. Based on this single paediatric PK study (n=20) the BNFC increased their dose recommendations to 10mg/kg either 8 or 12 hourly for infants >1 month age in 2011 [173]. This

higher dose has not yet been validated with subsequent PK, PD or safety data for infants or neonates. This emphasises the need for neonatal and infant PK data presented in Chapter 2.

To optimise treatment of more serious infections a higher $AUC_{24}$/MIC target of ≥250 is strongly supported by adult PD data. Historically the optimal predictor of efficacy targets a minimum $AUC_{24}$/MIC ratio >125 and this has informed clinical breakpoints [134, 162, 176, 178]. Forrest *et al.* found the median time to bacterial eradication significantly reduced to 1.9 days with an $AUC_{24}$/MIC ≥250 compared with 6.6 days when 125 - 250 and 32 days if <125 [162]. More recently in 2010, Zelenitsky *et al.* found higher cure rates (91.4%) were attained with a higher doses of 400mg 12 hourly resulting in an $AUC_{24}$/MIC ratio >250 when compared to 28.6% when <250 [176]. Lipman [115] recommended higher doses for more severe infection or pathogenic organisms in critical illness. The risk of adverse reactions from higher doses is relative to the risk associated with the severity of illness. Mortality in neonates with Gram-negative bacteraemia increased in this unit to 44% which indicates the need to target the higher ratio >250. Shortening the duration of bacteraemia is essential as prolonged hospitalisation is associated with increased morbidity and mortality [18]. The median (IQR) for days treatment with ciprofloxacin was 6 (2-10) range 1 – 37 days. Prolonged treatment may be due to under-dosing or, less susceptible organisms within the clinical breakpoint. These preliminary data are not conclusive but illustrate the need for ongoing evaluation of both PK and PD data specific to neonates.

The clinical outcome also depended on the type of organism. Neonates with bacteraemia caused by *Enterobacter* spp. were more likely to be cured (89%) compared to 44% with *E.Coli* even when the median and IQR of the MIC were similar Table 4-3. In contrast the MIC for *Pseudomonas* spp. was considerably higher than *E.coli* but the clinical outcome of the neonates was similar (Table 4-3). This suggests *E.coli* may be more pathogenic even when the MIC is lower. Fulminant late onset sepsis has previously been associated with

*Pseudomonas* spp. (42%) and *E.coli* spp. (10%) (lethal within 48 hours) [36]. In this cohort study *E.coli* spp., *Pseudomonas* spp. and *Klebsiella* spp. were the most common organisms associated with mortality in within ten days of confirmed Gram-negative septicaemia. Mortality from Gram-negative bacteria is mainly associated with antibiotic resistant bacteria (73%) [6]. Dose regimen may be based on the susceptibility when the organism is known. EUCAST's rationale document includes species specific breakpoints for adults and in some cases a higher dose or lower breakpoint is included for more serious infections [135]. *Acinetobacter* species is less susceptible than other Gram-negative bacteria yet the ECOFF (1mg/L) is higher than the non-species specific clinical breakpoint (0.5mg/L) therefore it is only classed as susceptible in adults when a higher dose is administered [138]. This confirms that breakpoints are specific to estimating the optimal outcome for a dose. These data suggest that the clinical outcome is determined by pathogenicity of the organism, the dose, the MIC and host response as is evident from other studies [36, 163, 295].

These critically ill patients were not a homogenous group. Surprisingly, the extremely premature babies with bacteraemia tended to have less treatment failure including the lowest PMA at birth (p=0.33) and lowest PMA at the time diagnosed (p=0.26) (Table 4-3). This may be associated with their immature renal function as the reduced excretion may have resulted in higher drug concentrations achieving a more optimal $AUC_{24}$/MIC ratio. This is plausible as ciprofloxacin is mainly excreted renally (70%) [296]. As with other antibiotics sub-age groups below 34 weeks PMA are reported to have slower excretion, including gentamicin and teicoplanin [126, 297, 298]. Teicoplanin concentrations were higher in neonates AUC 392 µg/h/mL than older children 221 µg/h/mL when administered the same dose per kilo [297, 298]. When a different regimen was administered to adults (400mg 12 hourly) this produced a higher AUC 550 mg/h/L [299]. This illustrates there is variability in AUC between neonates, children and adults yet the same breakpoint is applied even though different AUC in adults will result in different clinical breakpoints.

These data are consistent with the higher AUC found in more premature neonates in Chapter 2 (Figure 2.16). An alternative interpretation is that the more mature babies may have been exposed to a longer hospital stay with an increased risk of exposure to infection over time or other morbidity associated with a protracted illness resulting in a poor outcome. Again this illustrates that many factors not related to microbiology confound the clinical outcome in critical illness and rapid changes during neonatal development including co-administration of other antimicrobials, co-morbidity and effect of clinical interventions.

Outcome may be influenced by the host including the maturation of the immune system [36, 163, 295]. A higher proportion of male babies (64%) presented with Gram-negative sepsis but there was no significant difference in their clinical outcome Table 4-3. A gender bias is consistent with poorer outcomes as mortality is higher for male neonates globally 58%, yet the reason for this is unknown [300]. Equally, the clinical outcome may have been influenced by first line treatment with gentamicin or concomitant administration of co-amoxiclav. *Pseudomonas* spp*.* is dependent on susceptibility to either ciprofloxacin or gentamicin whereas *Enterobacter* are susceptible to co-amoxiclav [155]. Although these data are not statistically significant they are worthy of consideration to further understand the complexity of individual neonate's response to infection. This highlights the need for rapid identification of organisms and their susceptibility to antibiotics to individualise regimen.

Further safety data for different regimens is required to ensure there are no long term adverse reactions. The risk of an adverse reaction to a drug must be balanced against the severity of the outcome. As high mortality is associated with confirmed Gram-negative sepsis it may be more appropriate to increase the dose only when confirmed by a blood culture rather than suspected. Alternatively, the NTISS Score may be applied to classify a higher risk group. Individualised therapy may require titrating doses to the type of organism as some

bacteria are more pathogenic or for higher MIC. Safety data for dose regimen specific to each sub-age groups is required. Historically the main barrier to prescribing ciprofloxacin has been the potential risk of arthralgia/tendonitis [191]. A recent systematic review reported that musculoskeletal adverse events in children were reversible following withdrawal of the drug [190]. A brief episode of joint pain that subsequently resolves must be balanced against the risk of the outcome if 44% mortality Table 4-4.

### 4.4.3 Resistance

#### 4.4.3.1 Sub-clinical resistance - changes in MIC over time

Over a thirteen year period the annual incidence of bacteraemia was too low to determine whether sub-clinical resistance is emerging, a maximum of 15 isolates were available per annum (Figure 4-7). Few Gram-negative organisms in blood cultures were resistant (10%) (Table 4-2). The clinical outcome data found treatment failure was associated with higher MIC supporting the view by Falagas *et al.* and Labreche *et al.* that organisms classed as susceptible may need to be reviewed [67]

#### 4.4.3.2 Surveillance: relative resistance to ciprofloxacin and gentamicin

This Neonatal Unit's has an extensive surveillance programme to monitor throat and rectal surface swabs to identify epidemiologically significant organisms. Blood cultures are frequently collected (average 1000+- per annum) and routine surface swabs (average 6.9 per neonate). The incidence of ciprofloxacin resistance for each admission on surface swabs (0.99%) was no higher than gentamicin (1.31%) for non-duplicate resistant organisms (Table 4-6). The incidence of non-susceptible organisms on surveillance swabs was higher for *Acinetobacter* spp and *Pseudomonas* spp. yet less than 8 % for any species Figure 4-9.

There are limitations to using routine surveillance culture data to guide empiric therapy as neonates are colonised with many bacteria [42-44]. The aim is not to treat the patient for any bacteria identified in surveillance unless there are signs of suspected sepsis as this

would result in the over use of antibiotics and increase resistance. The main purpose is to identify resistant Gram-negative bacteria so that when a patient has signs of sepsis the early empiric therapy avoids antimicrobials that their surveillance swabs show resistance to in case the bacteria translocate . This rigorous surveillance is beneficial as a considerable proportion of Gram-negative isolates subsequently found in blood cultures (40%) were identified earlier on surveillance surface swabs Table 4 6. Early identification is valuable as these outcome data included four neonatal deaths subsequently found to have intermediate or resistant organisms in blood cultures. Clinicians may use surveillance data to predict the likely causative organisms prior to selecting antimicrobials and attempt to avoid those that likely bacteria are resistant to.

Antimicrobial resistance in adult intensive care patients are considerably higher. Neuhauser *et al.* evaluated 35,790 non-duplicate Gram-negative isolates in the US 1994 -2000 and found resistant *P.aeruginosa* 23%, *Enterobacter* spp. 14%, *Klebsiella Pneumoniae* 14% *E. coli* 11%. The authors related the decreased susceptibility of 10% over a six year period to increasing fluoroquinolone use [301]. The Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) programme demonstrated a decrease in susceptibility of fluoroquinolones over a ten year period. *E. coli* resistance rose rapidly from 4.1% to 31.8%, *Klebsiella Pneumoniae* from 4.2 % to 16.5% and *Enterobacteriaceae* 3.7 to 17.8% [302]. Frequent treatment with antibiotics may increase resistance particularly when administering broad spectrum antimicrobials [43, 46]. Suspected sepsis requires rapid treatment therefore many more neonates were treated than subsequently confirmed. Stoll *et al.* reported half of their cohort received antibiotics but only 21% subsequently had a blood culture proven infection [18]. In this unit approximately 30% of neonates are prescribed gentamicin (Badger patient data system) fewer are prescribed ciprofloxacin as this is often introduced as rescue therapy, yet the incidence of resistance for gentamicin and ciprofloxacin were similar. The over-use of antibiotics and in particular broad spectrum antibiotics causes an imbalance in gut flora that may subsequently lead to late onset sepsis

[43]. A higher proportion of resistant organisms were found in blood cultures (10%) than surveillance swabs (1.37%) (Table 4-2 and Table 4-6). This may be due to the fact that more resistant organisms have an increased risk of translocation from the gut flora as they are less effectively treated by early empiric therapy [43, 46, 47].

A review of antibiotic resistance concluded the highest tolerated dosing regimen must target the most resistant populations in an effort to prevent the emergence of resistant organisms due to selection pressures [48]. Achieving the mutant preventive concentration inhibits the least susceptible mutant within the wild type but this range is not usually targeted in clinical practice due to the lack of toxicity data [48, 164, 166, 303]. Higher doses may be required to prevent resistance. There may be an increased risk of resistance in paediatrics as antimicrobials are commonly prescribed unauthorised or off label. Inevitably the regimen is based on clinician preference rather than informed by reliable PK PD data. This is evident at there are wide variations in ciprofloxacin regimens internationally between 5 and 60 mg/kg/day [184]. Resistance may be fuelled by the resulting suboptimal regimen thereby allowing mutants to thrive.

The broadened anti-anaerobic spectrum of newer fluoroquinolones is thought to predispose patients to develop *C. difficile* [60]. McCusker *et al.* found an increased risk of *C. difficile* associated diarrhoea with ciprofloxacin 13.5 (3.1-58.8 $p<0.01$ compared to other antibiotics (1.6 0.7 -4.1 $p<0.28$) [304]. Rashid *et al.* monitored surface and faecal samples following a ten day course of ciprofloxacin to health volunteers and found no evidence of *C.difficile* [305]. Although infants have very high carriage rates of *C.difficile* in the gut it does not seem to cause disease in those < 2 years of age. This may be due to a lack of development of toxin binding receptor on the colonocytes of newborn infants [306]. Therefore *C.difficile* is not routinely monitored in neonatal surveillance.

### 4.4.4 Severity of illness score pre and post Gram-negative bacteraemia.

The change in illness severity when Gram-negative organisms were confirmed in blood cultures was quantified using the NTISS score. As anticipated those with more severe underlying illness at baseline were found to have a worse outcome (Table 4 3). These data illustrate a correlation between NTISS scores increasing on the day of isolation of Gram-negative organism compared to a baseline score two days previously. Equally, the neonate may have improved by the time septicaemia is recognised or responded to earlier treatment with antibiotics. An elevated NTISS score occasionally occurred two days before Gram-negative isolates were confirmed. This may be due to underlying illness, complications of prematurity, or delay between symptoms and the Gram-negative bacteria being isolated.

Illness severity scores are widely used in neonatal care. The NTISS Score was chosen for this study as it provides a more extensive detailed assessment of patients than other scores. It is unusual as it is mainly based on treatments administered rather than pathophysiological changes and treatment protocols may vary between hospitals [307]. The main limitation of this score in clinical practice is the extensive number of variables (n= 66) as compared to other scores such as CRIB (n=6) or SNAP (n=28) [307]. Even during a research study this is time consuming when case notes are extensive in critical illness. Bastos et al compared the four scores retrospectively and found comparable area under the ROC curve (for predicting in-hospital mortality) in each score was: CR18 0.90; SNAP 0.88; SNAP-PE 0.88; NTISS 0.85 [308]. However, there is no comparison yet of the ability of these scores in relation to detecting early signs of sepsis and the simpler scores may not have sufficiently variables to be sensitive.

The strength of the score is that in this retrospective study the illness severity score correlates to an objective laboratory measurement of confirmed organism in blood cultures. The value of these data is that they suggest the NTISS score may be a useful tool to

contribute to the clinical diagnosis of sepsis prior to the availability of confirmed cultures. The score may also be useful for randomised controlled trials either to assist in defining sepsis prior to laboratory confirmation or to stratify participants based on illness severity. Further assessment of the score including neonates with suspected versus clinically diagnosed sepsis would be valuable.

### 4.4.5 Clinical Implications and limitations

The strength of this study is that these data are clinically important. They provide insight to the clinical outcome and the susceptibility of Gram-negative organisms for a particularly vulnerable population with high mortality and morbidity associated with Gram-negative bacteraemia. Most isolates over a six year period were retained by the clinical laboratory and successfully re-cultured yet due to the low incidence of eligible neonates these data are neither statistically significant nor conclusive. However, there is a consistent trend suggesting the regimen was inadequate to optimise therapeutic success for severe sepsis as follows:

- A lower MIC within the susceptible range improved clinical outcome
- The MIC group >0.035 mg/L had a greater risk of treatment failure (RR 2.5)
- The pathogenicity of species influenced the clinical outcome
- Very premature neonates improved outcome may be associated with a higher AUC due to immature renal function or disease progression.

This retrospective outcome study provided an early indication of the need for a higher drug regimen in the absence of PK data specific to the population. The regimens at the time of this study (10mg/kg/day) may have been suboptimal to treat organisms with higher MIC. More recently this has been increased to 20 – 30 mg/kg/day based on infant and paediatric PK data supporting the findings from this clinical outcome study [115]. This crude model for comparing isolates from cultures with the MIC and the neonate's outcome may provide a framework for monitoring the effectiveness of regimen in the absence of conventional PK/PD data.

This study illustrates the limitations of assessing clinical outcome in neonatal sepsis. Even

in this large neonatal unit with >1200 admissions per annum the incidence of confirmed Gram-negative infection in blood cultures was relatively low 1.3%. The true number of neonates with bacteraemia may be higher. Frequent blood cultures are restricted due to the risk of anaemia, to protect veins and minimise distress. Equally, there is a risk of false negative results as small volumes of blood <0.5 mL are used which may be inadequate for sensitive and timely detection of bacteraemia. Schelonka *et al.* suggested 1-2 mL is required to recover microorganisms for low-colony-count sepsis [21]. Fewer than eighteen babies per annum were eligible for analysis and only a third were treated with ciprofloxacin for at least five days to meet the inclusion criteria (n=33) over a six year period. To achieve statistically significant data multi centre studies with international collaboration are required to represent each sub-age groups for both gestational and post natal ages and covariates associated with critical illness.

The challenges highlighted by this study partly explain why neonatal drugs are commonly unauthorised therefore prescribed off label more so than to any other patient population [309]. *In vitro* susceptibility testing of new antimicrobials usually requires several hundred isolates and at least ten organisms of each species to be tested [6]. To determine clinical breakpoints ideally requires several trials, and data sets with thousands of MIC. These neonatal data are limited to 33 patients with less than nine MIC values for each genus to compare with clinical outcome in this single centre. The outcome is confounded by the complexity of critical illness and clinical interventions therefore a randomised controlled trial would be required to evaluate safety and clinical outcome in this population. Yet this is difficult to achieve as to power a paediatric sepsis trial to detect a significant relative reduction in the risk of mortality using a baseline mortality of 10% as a primary end point would require 4000 babies [29]. There were insufficient patients at this single centre to compare the complex variables and host response for conclusive analysis.

The cure endpoint in this study may not be optimal, cure was defined simply as survival and CRP <10mg/L at day ten. This was chosen arbitrarily to reflect decision making in clinical practice. The advantage of selecting a short term outcome was in an attempt to determine a temporal association between the antibiotic and the outcome. Composite measures such as 30 day mortality or ventilator free days are confounded by death and morbidity from other causes in critically ill infants [231]. The time frame chosen for mortality resulted in different outcomes, short term mortality (within 10 days of confirmed Gram-negative organism) was lower (15%) when compared to mortality prior to discharge (44%). This illustrates the difficulty of selecting an outcome representing different stages of the admission. There is a need to standardise outcome of sepsis in neonate to allow evidence synthesis and meta-analysis. At present there is no consensus on standardised outcome measures specific to neonatal sepsis or published standards within the Core Outcome Measures for Clinical Trials initiative (COMET) [29, 231, 310].

Individualised therapy may improve the outcome of critically ill neonates by applying personalised antimicrobial strategies. Higher doses may be prescribed for organisms found to be more resistant even within the susceptible range, those that cause severe infections including *E.coli*, *Pseudomonas* spp. and *Klebsiella* spp. or high risk sites such as bacteraemia or meningitis. This is dependent on the knowledge of the exact organism and MIC when antimicrobial therapy is commenced. In clinical laboratory practice the E Test is not routinely used as it is expensive and results are not available for a minimum of 24 hours incubation period. Newer rapid methods for identifying the organism at species level include MALDI-TOF-MS a mass spectrometer and real time PCR assays may resolve this [311]. Resistance may vary locally depending on antibiotic usage and infection control practice therefore monitoring MIC's at each site is recommended. Further safety and PK data is required to determine the standard and maximum effective dose that can be tolerated without significant adverse effects [79].

Clinical laboratories throughout Europe determine the susceptibility of organisms by estimating which MIC is most effectively treated by an adult regimen and outcome data. At present there are no clinical breakpoints specific to neonatal or paediatric PK-PD data. EUCAST's breakpoints are mainly informed by three variables i) drug regimens ii) clinical outcome (specific to the adult population) iii) MIC of the organism (Figure 1-21). Any change to a variable other than the MIC in particular the dose, site of infection or population will alter the predictability of the MIC to achieve therapeutic success. In contrast to the ECOFF which is the concentration required to inhibit bacterial growth in a laboratory. Ciprofloxacin clinical breakpoint predicts the MIC most likely to be effectively treated by a regimen 500mg 12 hourly (oral) or 400mg x 12 hourly (intravenous) [159]. As the neonatal drug regimen varies not only from adults but between neonatal units internationally, therefore the application of the same breakpoint is unlikely to predict success. The AUC/MIC ratio is the optimal predictor of cure for Ciprofloxacin and different antimicrobial regimen result in different AUC as shown when comparing Teicoplanin PK data for an adult compared to either a child or neonate [155, 297, 298]. There is an established evidence base that drug regimens for adults cannot be extrapolated to children [79, 80] similarly the outcome of an adult drug regimen cannot be extrapolated. Whilst these neonatal data are insufficient to inform a clinical breakpoint but indicate that a dose of 10mg/kg/day was only sufficient for organisms with a MIC of 0.035mg/L. To some extent the interpretation of these data are supported by the fact that the dose was subsequently increased based on PK data for older infants. This retrospective non-invasive method for monitoring neonatal outcomes compared to the MIC of the causative organism may provide a framework for identifying sub-optimal treatment regimen.

Further data are required. These data are insufficient to determine the optimal clinical breakpoint but illustrate that the optimal MIC treated by a neonatal regimen is unknown. This study led to the hypothesis that neonatal and paediatric microbiological breakpoints

informed by PK, safety and PD data for each sub-age group are required to optimise outcome. Different breakpoints may be required for sub-age groups as antimicrobial regimen are prescribed on weight (mg/kg) and maturation may influence the individual's response. This issue has not been published previously. Historically, the lack of PK /PD data for children has prevented the licensing of medicines but since the EU Paediatric Regulations 2006 [73] the increase in paediatric research may now provide the evidence base for a paediatric breakpoint model. To obtain sufficient data collaboration of neonatal units throughout Europe is required such as the consortium funding this ciprofloxacin study (Treat Infection in Neonates www.tinn-project.org).

### 4.4.6 Conclusion

In conclusion, there was a low incidence of confirmed Gram-negative organisms in neonatal blood cultures but when present this was associated with considerably higher mortality. A lower ciprofloxacin MIC was associated with likelihood of cure among Gram-negative strains in blood cultures that were nominally susceptible to ciprofloxacin. Infections with susceptible strains that have relatively high MIC values or those known to be more pathogenic may need dosage optimisation. Despite long term use of ciprofloxacin in this unit there is no evidence of rapid resistance developing. Individualising drug regimens based on the MIC, pathogenicity and anticipated host response of the organism may optimise therapy. In the absence of classic PD data this non-invasive retrospective PD model developed in this research has value in identifying suboptimal treatment regimens. An evaluation of neonatal and paediatric clinical breakpoints informed by PK, safety, microbiological and clinical outcome data is required to guide clinical practice.

# Chapter 5 Regulatory Model: Neonatal PK Clinical Trials

## 5.1 Overview

Neonatal clinical trials are possibly the most stringently regulated research as there is additional provision in the regulations to protect this vulnerable group [233]. The regulatory requirements are more rigorous for PK clinical trials as they are commonly for unlicensed or off label drugs. Neonatal PK data are scarce therefore data reliability is essential as the results have considerable influence on prescribing globally. The Clinical Trials Directive applies to all clinical trials of medicinal products in Europe, from “first in man” trials to pragmatic comparisons of commonly used treatments yet made no distinction between them with regard to the GCP requirements [232]. The Academy of Medical Science Report regarding the regulation and governance of health research stated the regulatory framework is a barrier to trials [235]. In response to this the MHRA, DOH and MRC prepared a consultation paper to develop a more proportionate approach. This Chapter describes a model for PK trials in neonates following a direct request by the MHRA.

The request was to develop a risk assessment tool with guidance principles on how to manage and conduct CTIMP in a risk proportionate way. The research fellow developed a bespoke model with methods to mitigate risk to the safety of participants and reliability of data. The model was validated by the national steering group appointed by the DOH led by the MHRA including national stakeholders for the National Research Ethics Service, GCP Inspectorate, Pharmaceutical Industry, MHRA Medical Assessors and Academics and NHS Research Governance Leads. To engage with international Competent Authorities the research fellow presented the model at an international meeting with the EMA and FDA drug development leads. The model is published on the MHRA web site.

### 5.1.1 Aim and Objectives:

To develop a regulatory model for neonatal PK clinical trials within the MHRA Consultation process more proportionate to the Investigational Medicinal Product.

**Objectives:**

To reduce regulatory requirements and barriers whilst mitigating potential risks to the safety of participants and reliability of trial data

To risk assess the investigational medicinal product (IMP) relative to ciprofloxacin's marketing authorisation relative to use in standard medical care for neonates and infants.

To summarise EU and UK Regulatory requirements relevant to clinical trials then identify adaptations proportionate to the risk of the IMP.

To design a bespoke trial model for a PK clinical trial with methods to mitigate potential risks related to the design and methods of the trial.

To validate the model within a national consultation process with experts within the national steering group chaired by the MHRA.

To assess the impact of reduced regulatory requirements for a PK clinical trial in neonatal and paediatric intensive care.

To illustrate:

- The incidence of adverse events in critical illness
- The complexity of determining causality

To develop a safety reporting framework proportionate to the complexity of causality in critical care.

## 5.2 Methods

The MHRA Consultation process for a risk proportionate approach to clinical trials was the outcome of a risk-stratification project initiated by the Department of Health, MHRA and Medical Research Council to address key issues for clinical trials in the UK [232]. . The current regulatory framework in the UK/EU allows for a range of risk-adapted approaches largely related to how much is known about the investigational medicinal product (IMP). A simple risk categorisation based on the marketing status of the IMP and standard medical care determined the lower risk trials, where simplification of regulations results in a more risk proportionate approach. Three risk categories were proposed in the MHRA Consultation Document:

**Type A** – comparable to or no higher than authorised use or use in established standard care,

**Type B** – somewhat higher than licensed use for example a new indication or dose modification

**Type C** – Markedly higher than the risk of standard medical care

### 5.2.1 Risk assessment of ciprofloxacin specific to neonates and infants

A risk assessment process is proposed to identify potential vulnerabilities in trial design and methodology, and to prepare a trial management and monitoring plan to minimise the risks. Risk was defined as the likelihood of a potential hazard occurring and resulting in harm to the participant and/or an organisation, or to the reliability of the results [232]. A risk assessment was undertaken for the IMP ciprofloxacin administered to neonate and young infants relative to the marketing authorisation. Ciprofloxacin is unauthorised therefore prescribed off label to neonates in the MHRA public safety assessment report [53, 173]. The MHRA Medical Assessor then evaluated the risk of the IMP relative to use in standard care. This was a summary of published evidence reporting the administration of the ciprofloxacin to neonates in standard care including neonatal and infant prescribing guidelines in the BNF for Children and publications including systematic reviews and surveys.

The risk assessment evaluated 'the likelihood of a potential hazard occurring and resulting in harm to the participant, the sponsoring organisation or reliability of the results' [232]. The safety profile of known adverse reactions was summarised from the Summary of Product Characteristics (Bayer PLC www.medicines.org.uk/emc/history/22508 accessed 2015.

### 5.2.2 Regulatory requirements proportionate to the risk of the IMP

The EU and UK Directives and Regulations relevant to CTIMP in paediatrics were summarized. Potential adjustments proportionate to the risk category Type A, B or C of the Investigational Medicinal Product were determined for the following activities:

Table 5-1 Adaptations based on the Risk

| | **Increasing Risk** → | | |
|---|---|---|---|
| **Risk Adaptations Possible** | **Type A** | **Type B** | **Type C** |
| Reduced role of competent authority approval for clinical trial authorisation and the content | Yes | No | No |
| Content of Application | Yes | (Yes)* | No |
| Investigational Medicinal Product management | Yes | (Yes)* | (Yes)* |
| Labelling | | | |
| Storage | | | |
| Drug accountability | | | |
| Safety Surveillance | Yes | (Yes)* | No |
| Documentation | Yes | (Yes)* | No |
| GCP Inspections | Yes | (Yes)* | (Yes)* |

*On a case by case basis

### 5.2.3 Design a bespoke clinical trial model with methods to mitigate risks.

A bespoke clinical trial model was developed with a risk assessment of:

- Sponsorship and governance
- Investigational Medicinal Product
- Patient consent rights and confidentiality
- Reliability of Trial Results
- Facilities, equipment and resources
- Documentation, Governance and GCP Compliance

Strategies to mitigate each risk incorporated local or national standards for neonatal and paediatric clinical practice. Methods to mitigate risks were integrated in the protocol and standard operating procedures.

### 5.2.4 Validation of the model

The model was submitted to the MHRA National Consultation Group for publication on the MHRA web site. During face to face discussions at the MHRA Head Quarters each regulation and methods to mitigate risks were examined by the steering group including:

- MHRA Good Clinical Practice (GCP) Inspectors
- MHRA Medical Assessor
- National leads for the pharmaceutical industry
- Academic researchers
- National Research Ethics Service
- NHS Research Governance Managers

### 5.2.5 Framework for safety reporting proportionate to critical care

To extend the model a pharmacovigilance framework was designed proportionate to the high incidence of adverse events and complexity of determining causality in critical illness. The aim was to minimise reporting events that could not be directly associated with causality without a RCT thereby avoid nuisance reporting. The nature of intensive care means that events are frequently but associated with critical illness. Adverse events were defined as ‘any untoward medical occurrence in a subject to whom a medicinal product has been administered including occurrences which are not necessarily caused by or related to that product’ [245].

To illustrate incidence of adverse events in a critical care setting events reported in the discharge letters were summarised to illustrate the frequency of events in critical illness. Serious and non-serious adverse events that the clinician considered to be clinically important were summarised for 20 neonates recruited to the PK clinical trial. To illustrate the complexity of causality assessments an observational case study of a critically ill infant admitted to paediatric intensive care included a level IV patient representing the most

severe illness based on the Paediatric Intensive Care Society definition [312]. The research fellow trained in paediatric intensive care and a trial pharmacist observed the patient over a seven hour period. Potential triggers for each adverse event were identified that may have contributed to the event including:

i) clinical interventions
ii) clinical condition
iii) concomitant medications

Baseline values for vital signs and ventilator observations were agreed for the patient's stage of development with an Intensivist and two senior PICU nursing staff. Baseline laboratory reference ranges were consistent with the PK clinical trial protocol.

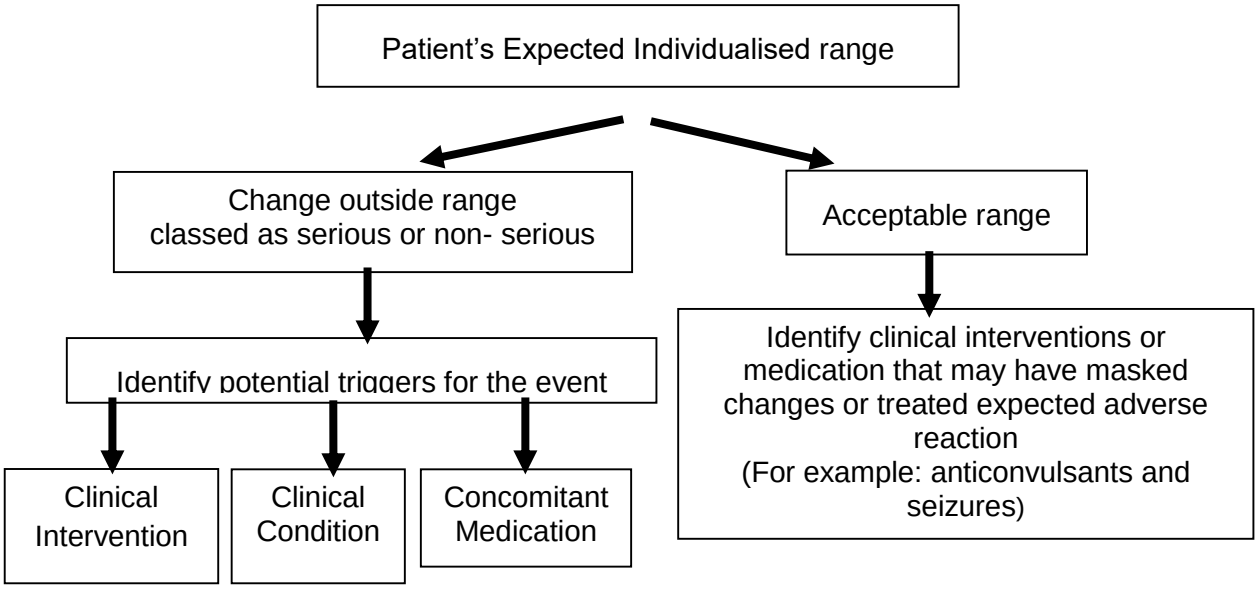



Figure 5-1 Framework for assessing Adverse Events in Critical Care

This figure illustrates the method for assessing potential adverse reactions masked by the clinical interventions or concomitant medication

## 5.3 Results

This Risk Proportionate Approach to the regulations for PK clinical trials was implemented by the MHRA in 2013 [232]. This model for neonatal and paediatric clinical trials met the Department of Health deadline and was published as a guide to investigators on the regulatory web site http:// forums.mhra.gov.uk/forumdisplay.php?22-Risk-adaptive approach. The full model is included in appendix V.

### 5.3.1 Risk Assessment of Ciprofloxacin specific to neonates and infants

The Marketing Authorisation confirmed that ciprofloxacin is prescribed off label for neonates: it is licensed in children >1 year age for infections where the benefit is considered to outweigh the potential risks of the drug [53, 173]. The MHRA medical assessor initially classed the IMP as Type B as the IMP did not have a marketing authorisation when prescribed to neonates. A review of evidence supporting the use of ciprofloxacin in standard care was summarised including a systematic review of ciprofloxacin in neonates and a European survey funded by the TINN Consortium (Table 5-2)[73]. MHRA were satisfied with this evidence and revised the risk classification as Type A (low risk). The lower risk was supported by the fact that the inclusion criteria required ciprofloxacin to be prescribed as standard care therefore the risk of participation related to additional monitoring (blood samples) rather than administration of an IMP.

Table 5-2 Evidence of Ciprofloxacin prescribed to neonates in standard care

| | |
|---|---|
| **Clinical Trial Protocol** | IMP is prescribed by the clinical team as standard care (not for the purpose of research) |
| **Prescribing Guidelines** | SmPC and MHRA Public Safety Report include the indication severe infection [53]<br>British National Formulary for Children includes off label prescribing guidelines for neonates [173]<br>Liverpool Women's NHS FT Neonatal Sepsis Protocol |
| **Published evidence** | Systematic review of adverse events in children (105 articles and 16 184 patients) [190]<br>Systematic Review of the use of ciprofloxacin in neonates (32 cohort studies or case reports [184]<br>Ciprofloxacin in neonatal *Enterobacter* spp*. cloacae* septicaemia *confirming use since1989* [313]<br>Improving antibiotic prescribing in neonatal units – time to act - [183] |
| **European Survey** | 26% (50/193) units prescribe ciprofloxacin as first or second line antimicrobial therapy http://www/tinnproject.org |
| **Off label use** | Off label use in Europe of established practice for children and neonates – 55% of drugs are administered off label to neonates [309] |

### 5.3.2 Regulatory requirements proportionate to the risk of the IMP

EU and UK Regulatory requirements applicable to a PK clinical trials for neonates were reviewed then adjustments proportionate to the Type A risk category were summarised

Table 5-3 Regulatory requirements for a Type A trial of ciprofloxacin in neonates

*** Regulation refers to the Medicines for Human Use Clinical Trials Regulation [245] and subsequent amendments**

| Regulation* | Reduced Regulatory Requirement for the Neonatal PK Trial |
|---|---|
| **MHRA Authorisation** | Exempt from a Clinical Trial Authorisation ( automatically approval) [232] |
| **Content of Application** | SmPc replaced the IMP dossier and Investigator Brochure [232].<br>Substantial Amendments are not required when the protocol change was consistent with the SmPC. |
| **IMP Management** | Prescribing standards within the NHS allowed exemption from:<br>Drug accountability log (of expiry dates and batch numbers)<br>Labelling of dispensed drugs specifically for the trial<br>Temperature monitoring of ward stock |
| **Safety** | Serious Adverse Events anticipated in critical illness including death were exempt from expedited reporting<br>Non-serious adverse events /reactions were limited to those identified by the Sponsor as critical to the safety of the trial.<br>The period of reporting during follow up was limited to 42 days post administration of the drug rather than the end of the trial. |
| **Consent, rights and confidentiality** | Consent was delegated to medical staff and registered nurses with relevant generic research training and study specific training who appeared on the delegation log [234] (not restricted to medically qualified personnel)<br>Emergency deferred consent allowed the blood sample to be collected prior to consent Amendment 2984 [240] |
| **Monitoring and Inspections** | Site monitoring visits were higher based on the risk assessment for data reliability<br>Frequency of MHRA Inspections was reduced for Type A |
| **Documentation, Governance and GCP** | Reduced content of trial master files was allowed at follow up sites using a simplified electronic site file specific to the role of the site (limited to the protocol, statement of responsibilities and site specific standard operating procedures relevant to follow up). |

### 5.3.3 A bespoke clinical trial model to mitigate risks

A detailed risk assessment and methods for mitigating the risk were referenced to the appropriate regulations Appendix V. The following areas of risk were identified:

- Sponsorship and Research Governance
- Investigational Medicinal Product administered to neonates
- Safety
- Patient Consent, Rights and Confidentiality
- Reliability of Trial Results
- Facilities, Equipment and Resources
- Documentation, Governance and GCP Compliance

The risk assessment categorised the likelihood of each potential hazard as low medium or high and the impact (see appendix V). The risk of the IMP to patient safety was assessed as low risk as the drug was administered as part of standard care not for research.

**Three high risk categories were identified all associated with precision and data reliability:**

- **Drug administration**
- **Drug accountability**
- **Blood Sampling**

#### 5.3.3.1 Drug administration Risk of precision – High Risk

This quality of the data and the validity of the trial were classed as a high risk to achieve the level of precision required for a PK trial. Potential sources of variation were determined. The risk to precision depended on how the infusion was set up either primed with saline first or with the drug. The risk was higher for the low weight babies partly due to the fact that the volume of drug was less therefore took longer to reach the vein when administered via long infusion lines. At the paediatric site the drug could be delayed from reaching the vein for up to 24 minutes as the dead space between the patient's vein and the drug could be up to 2 mL and the drug was administered over 60 minutes.

There is a risk that not all the drug enters the circulating blood prior to taking the sample. As the internal length of neonatal long lines and Broviac lines could not be reliably estimated. This posed a particular risk for the group A sampling schedule as T1 was taken within 3 minutes of the end of the infusion. The flush volume was restricted for critically ill babies particularly those with cardiac defects to 1mL/kg/hour. In a neonate weighing 1kg their total allowance is 1 mL per hour. Small flush volumes can be a considerable proportion of their daily intake particularly when administering multiple drugs. These patients are often administered >20 drugs per day. Each mL results in a decrease in the baby's nutritional allowance.

#### 5.3.3.2 Risk of drug accountability – high risk

The requirement for trial specific drug stock accountability logs, labelling and temperature monitoring was avoided as the drug was prescribed and dispensed within the NHS [232]. The drug could be dispensed from ward stock. The impact was considerable during this PK trial as over 500 intravenous infusions of ciprofloxacin were administered. The burden was reduced for clinical staff as they were not required to collect a trial supply from pharmacy. Equally this avoided the risk of delaying administration as the neonatal antimicrobial policy states antibiotics should be administered within thirty minutes. Potential patients may have presented on any of seventeen paediatric and neonatal wards in two lead centres. The proportionate approach reduced the burden of monitoring the temperature of drug stock in each area and the cost of purchasing minimum/maximum thermometers. To monitor the temperature of the ward stock of trial drugs in each of these areas would have taken over two hours a day. Neonatal units are often warmer than the recommended temperature for storing this drug. To mitigate this risk unpublished data from the pharmaceutical company was obtained stating that ciprofloxacin is stable up to 40˚C.

#### 5.3.3.3 Risk and burden of blood sampling – high risk

The population PK design reduced the risk of frequent sampling as it allows individual patients to contribute sparse samples rather than rich sampling from a single patient. The HPLC MS laboratory method was developed to measure drug concentrations on small plasma volumes 0.2mL. DNA samples were scavenged from blood that was taken for clinical care if sufficient remained following the clinical analysis. A sub-study confirmed that DNA could be scavenged post transfusion without donor contamination affecting the genotyping as blood products are now leucocyte depleted (Chapter 3). Blood samples were obtained within the guidelines on the total volume of blood collected from a child for research [80, 140].

#### 5.3.3.4 Methods to Mitigate Risks

To optimise the precision of drug administration and sampling it was essential to train all the clinical staff as data was required at any time day and night. The research fellow provided approximately 50 training sessions day and night to >300 clinical staff including medical, nurses, pharmacists, laboratory and phlebotomists. Precision of the data depended on accurate clinical records being completed by each clinical nurse including the method of priming an infusion line, start and end times. Standard operating procedures were prepared based on the risk specific to each recruiting site as different infusion practices were in place. Trial tools including recruitment packs were prepared with the exact information needed for the bed side nurse. The research fellow attend the neonatal unit each shift that either a sample was due to remind staff of the precision required and to provide the patient labelled blood or CSF bottles or swabs etc. To minimise distress from sampling each sampling schedule was planned individually to coincide where possible with clinical blood gas or laboratory samples. The frequent clinical sampling provided an opportunity to minimise the distress and blood loss for PK analysis.

On site monitoring of sources verification data was undertaken by the Sponsor based on this risk assessment [245]. Serious Breaches that may have affected the scientific integrity of the trial were reported to the Sponsor as per Regulation 29A [233]

### 5.3.4 Validation of the model

The model was validated initially by two Senior GCP Inspectors and the MHRA Medical Assessor. They examined the model to ensure the model was consistent with the regulations [233, 245, 314]. The final approval was a face meeting with stakeholders including the national consultation group members. The model was published on the MHRA website in fulfilment of the requirement by the Department of Health. Each clinical trial regulation was examined in detail to ensure the adaptations proposed in the risk

proportionate approach were consistent with the UK regulatory framework and that potential risks were mitigated.

#### 5.3.4.1 Evaluation of a pharmacovigilance framework proportionate to critical care

A safety reporting framework was developed proportionate to the high incidence of adverse events and complexity of determining causality. SAE anticipated in this population were defined in the protocol based on the incidence of conditions in very premature babies (Prof Cooke personal communication). Whilst these were reported they were exempt from expedited reporting to the Sponsor Regulation 32 (4) [245] . This reduced the pressure for a Principal Investigator to report the event to the Sponsor within 24 hours. The incidence of SAEs is monitored by the Sponsor and Independent Safety Data Monitoring Committee (ISDMC). The reporting interval is determined by a risk assessment. The incidence was compared to what was anticipated (associated with the health condition) or expected (associated with the drug based on the reference safety information (RSI)). If this increased the PI must notify the MHRA and National Research Ethics Service of a Suspected Unexpected Serious Adverse Event (SUSAR). In this way the safety of the patient is protected without the burden of reporting within 24 hours. The rational is that a decision to stop or changing the trial protocol would only be made if there was an increase in the incidence not an isolated event. During this trial there were seventeen serious adverse events. These were subsequently assessed by independent clinicians as having no evidence of a causal link and were exempt from expedited reporting.

In critically ill patients non-serious adverse events occur frequently. Regulation 32 (5) allows the Sponsor to define the non-serious adverse events or reactions ‘critical to the safety’ of the trial or of interest to the outcomes that require reporting in the protocol. A risk assessment of the IMP identified adverse reactions previously reported in the SmPC, MHRA public assessment report and systematic review on safety in children with an

estimate of the likely hood of the event occurring Table 5-4. These non-serious adverse events were collected systematically in the case report form and summarised in safety reports for review by the ISDMC and the Sponsor. This reduced the burden for investigators completing reports for events that were not required and ensured that any event of interest was systematically collected.

Table 5-4 Expected adverse reactions listed in the ciprofloxacin SmPC

| **Body System** | **Adverse reaction/Hazard** | **Likelihood** Rare, Low, Medium or High |
|---|---|---|
| Joints | Arthropathy/ tendonopathy | M |
| Vein | Phlebitis | L |
| Liver Function | Failure /Pancreatitis | L |
| Renal Function | Failure /Crystalluria | L |
| Blood cells | Deranged | L |
| Gastro-intestinal | Colitis if severe and persistent *Clostridium Difficile* | M |
| Anaphylaxis /Allergy | Shock | R |
| Neurological | Convulsions | M |
| Cardiac | Ventricular arrhythmias /Prolonged QT interval | |
| Skin | Rash/ Photosensitivity | M |
| Syndromes | Stevens Johnson /Lyell | R |

The period of pharmacovigilance reporting was specified as a set time of six weeks after drug administration supported by Regulation 32(5) Section 3 MRC /DOH Clinical Trial Tool Kit [245, 315]. Open statements are often used in protocols such as last patient ‘last visit’ yet the trial may last several years imposing considerable burden on the researcher. As neonates are often readmitted to hospital for other reasons associated with prematurity this would require frequent further reports for the duration of the trial of admissions to other hospitals not just the recruiting site. Limiting the reporting period in the protocol avoids unnecessary reporting and is supported by the Clinical Trial Tool Kit [315]. By defining a time in the protocol means the safety reproting peroid is approved by the MHRA and Ethics as being a sufficient period to record the safety outcomes.

**Serious Adverse Events or Reactions (SAE /SAR)**

Anticipated SAE: Sponsor specifies SAE anticipated in this patient population in the protocol
Unanticipated SAE: All SAE are reported but are exempt from expedited reporting within 24 hours

SAE that are not anticipated in the protocol must be reported by the Investigator immediately (usually within 24 hours) of their knowledge of the event to the Sponsor Regulation 32 (4)

↓

**Suspected Unexpected Serious Adverse Reaction (SUSAR)**

If the incidence of SAE increases above the anticipated rate the Sponsor expedites a SUSAR report to the MHRA/NRES/competent authority of other member states involved)
SUSAR that are fatal or life threatening' must be reported within 7 days after being aware of the reaction or 15 days if not fatal or life threatening. Regulation (33)

↓

**Non-serious Adverse Events / Reaction Reports**

The Sponsor defines the non-serious AE/AR that are 'critical to the safety' of the trial in the protocol if they require reporting. This is based on the known reactions for the group of drugs in the SmPC Regulation 32 (5)
Investigators may report any other adverse reactions when causality is suspected particularly if this is not a known adverse reaction.

↓

**Monitoring Non-serious Adverse Events and Reactions defined by the Sponsor**

Non-serious adverse events may be systematically recorded in the case report form depending on the urgency of reporting based on the Sponsor's risk assessment.
Case report forms must capture clinical interventions, concomitant medications or clinical conditions that may confound the assessment of causality

↓

**Limit the period of pharmacovigilance reporting**

Define the period of safety reporting following the administration of the drug to that patient rather than an open statement 'last patient last visit' Regulation 32 (5)

↓

**Annual Safety Report**

The Sponsor provides an Annual Safety Report for the REC and MHRA (competent authority of any member state) taking into account all new available safety information received during the trial (Regulation 35)

Figure 5-2 Pharmacovigilance reporting framework proportionate to critical care

#### 5.3.4.2 Evaluation of Adverse Events in critical illness

##### 5.3.4.2.1 Adverse events in neonatal discharge letters

A retrospective review of discharge letters assessed the number of reports that met the regulatory definition of non-serious adverse event or reaction. Among the first twenty neonates recruited to the clinical trial an average of 11.3 adverse events were reported Table 5-5. Serious Adverse Events that occurred during the six week follow up period included eight deaths and six other SAE. Most SAE were exempt from expedited reporting to the Sponsor (93%) as they were pre-defined as anticipated in this population due to prematurity and the incidence stated in the protocol. One SAE had not been listed as anticipated but subsequently classed as no causality). Three non-serious adverse events relating to renal calculi had possible causality. The Independent Data Monitoring Committee found this was likely to be associated with other factors such as administration of diuretics. Causality for adverse reactions was confounded by the fact that each baby was administered an average of 18.8 concomitant medication during their admission.

Table 5-5 All cause AE /AR reported in Discharge Summaries for Neonates (n=20)

| **Adverse event /reaction** | **N=** | **% of babies** |
|---|---|---|
| Respiratory Distress syndrome | 17 | 85 |
| Jaundice | 16 | 80 |
| Anaemia | 14 | 70 |
| Thrombocytopenia | 14 | 70 |
| Hyponatraemia | 13 | 65 |
| Intraventricular haemorrhage | 10 | 50 |
| Bronchopulmonary Dysplasia | 10 | 50 |
| Metabolic Bone Disease | 9 | 45 |
| Necrotising enterocolitis | 6 | 30 |
| other | 126 | n/a |
| **Total** | **226** | |

**Average** 11.3 adverse events or reactions per baby

5.3.4.2.2 Adverse Events: Paediatric Intensive Care Infant: observational case-study

To illustrate the complexity of assessing causality for adverse events an infant diagnosed with Meningococcal Septicaemia was observed to represent a most severely ill category level IV of Intensive care patients. Adverse events anticipated with the clinical condition for meningococcal septicaemia include:

- Disseminating intravascular coagulopathy (DIC)
- Systemic Inflammatory Response Syndrome (SIRS)
- Multi System Organ Failure (MSOF)

Fifteen non-serious adverse events or reactions were identified over a seven hour period that met the regulatory definition of ‘any untoward medical occurrences’ (estimated as 360 reports per week) (Table 5-6 ). These included changes to vital signs, blood values, ventilation parameters and blood gas analysis. This illustrates how these factors are equally associated with their clinical condition, clinical interventions and concomitant medication confound the assessment of causality. This patient was administered 14 concomitant medications during the seven hour period including seven continuous infusions. Interventions to treat low blood pressure included inotropes, human albumin solution or intravenous fluid would mask hypotension associated with an anaphylaxis to the IMP. As the patient was haemofiltered this renal support would mask potential adverse reactions.

Table 5-6 Potential Triggers Confounding Causality of Adverse Events

| Adverse Event /Reaction | Clinical Condition | Clinical Intervention | Concomitant Medication |
|---|---|---|---|
| **Vital signs**:<br>Tachycardia<br><br>Hypotension<br>Core Temperature ↑↓ | Yes (all) | Physiotherapy /Endotracheal Suction<br>Haemofiltration (negative balance)<br>Active warming<br>Oesophageal temperature probe (risk: detects external heat<br>Haemofiltration circulating temperature | Inotropes - adrenaline, nor-adrenaline, dopamine and milrinone<br>Opiates (Fentanyl)<br>Temperature masked by paracetamol |
| **Blood Values**<br>Anaemia<br>Thrombocytopenia<br>AST ↑ ALT ↑<br>Derranged clotting<br>↓Bilirubin<br>↑Albumin<br>↑C-reactive protein<br>Hypoglycaemia | Yes (all) | Blood loss from sampling, central line insertion or priming haemofiltration circuit<br><br>Anticoagulants for haemofiltration<br><br>Naso-gastric or intravenous nutrition delayed or inadequate | Fluid resuscitation<br>Human Albumin Solution (over correction)<br><br>Heparin in the circuit |
| **Respiratory compromise**<br>↓ tidal volume<br>↓ respiratory rate<br>Acidotic | Yes (all) | Ventilation rate masks respiratory depression<br>Endotracheal tube blocked with secretions | Opiate associate respiratory depression Fentanyl or sedation Midazolam<br>Fluid Resuscitation |

## 5.4 Discussion:

Despite the fact that this is a vulnerable neonatal population, this model has demonstrated that a regulatory risk proportionate approach may be applied to trials involving off label medicines to neonates. A bespoke trial management plan including methods to mitigate potential risks avoids compromising the safety of children or data reliability in intensive care. The MHRA considered the use of off label drugs that are commonly prescribed in standard care as low risk rather than restricting the category to the marketing authorisation as it is common in paediatrics for medicines to be administered off label [309]. It is evident that the highest risk for this PK trial was data reliability which has considerable implications as the data will influence prescribing regimens internationally.

Monitoring the safety for critical care patients has challenges. As this patient population are clinically unstable they present with a complex series of adverse events anticipated by the nature of their condition. Pharmacovigilance reporting can be a burden to busy clinical teams and result in a vast amount of nuisance reporting of events that are either not related or possible to know in this design. In the absence of an event with a temporal association to the intervention causality could not be reliable out with a randomised controlled trial. The burden to busy clinical teams is immense. The observational case study suggests as many as 360 non-serious adverse event reports would be generated within a seven day period for a critically ill PICU patient. The retrospective review of discharge letters found 11.3 adverse events per neonatal admission. A proportionate approach to safety reporting provides a pragmatic solution for clinical teams that limits pharmacovigilance reporting to SUSAR, SAE and non-serious AE that the sponsor has identified as being crucial to the participant's safety.

Safety reports risk inferring causality without proven association with the IMP. Adverse events commonly associated with infection were reported in the systematic review of ciprofloxacin safety in paediatrics [190]. These adverse events are commonly anticipated as part of the clinical condition therefore this review may mislead clinicians and regulators. The review did not provide a separate analysis of adverse events identified in randomised controlled trials therefore it was not possible to reliably determine causality in respect of ciprofloxacin administration.

Determining causality in critical illness is complex as the clinical condition, clinical interventions and concomitant medications confound an assessment of the IMP. For example in the observational study of meningococcal sepsis deranged clotting is anticipated due to the illness and therefore difficult to determine when causality is due to the IMP. In this PK clinical trial neonates were administered on average eighteen medicines during their admission. Adverse reactions listed in the SmPC are often common to more than one medication. Equally, clinical interventions and medication that support organ function may mask adverse reactions such as inotropes masks hypotension and ventilation masks respiratory depression. As such safety data from intensive care patients may not be generalisable to other patients who may then be at risk when organs are not supported. Despite this the intensive care environment provides a unique opportunity to observe trends following the administration of a drug due to the continuous monitoring and skilled nursing staff. This may allow the identification of temporal changes that may not be observed in other populations.

This study illustrates opportunities specific to critical care that can mitigate risks of research and overcome ethical challenges. The frequent clinical blood sampling during critical illness allowed the PK samples to be taken at the time of clinical blood samples preventing additional distress to the baby. Scavenging clinical samples from clinical blood samples prevents further blood loss for research by reusing blood that

would otherwise be discarded. However, this required a rigorous training programme inclusive of almost every member of the clinical team to ensure precise timing of infusions and sampling during especially as most drugs are given and samples taken during routine care. Scavenging clinical samples has the potential to collect *a priori* data to inform an optimal sampling schedule for future PK models.

Consent from parents was required to obtain samples. Prospective consent was used to achieve the tight time frame (3 minutes following the first infusion) as the drug was prescribed at any time of day or night. Parents often lived at a distance and have other siblings to care for so they are not always available. Requesting prospective consent allowed parents more time to consider participation. Also, they could be approached at a less stressful time. UK legislation allows nurses to be delegated the role of which meant information could be given to parents by the wider team at the children's hospital where there were >20 wards. At a later stage Emergency Deferred Consent and telephone consent were approved by the National Research Ethics Committee for this trial but rarely applied [240]. Legislation allowing emergency deferred consent is approved by amendments to the Medicines for Human Use Clinical Trial Regulations [240, 244, 245].

Follow up of neonates is challenging as they tend to move to another hospital nearer home across the neonatal referral network often up to 100 miles in case the baby was transferred [316]. Safety data was required for a six week follow up period , this required R&D approvals at 40 hospitals and a GCP trained Principal Investigator [317]. As this population were critically ill only 3/65 babies were transferred during the follow up period. It is difficult to predict which hospital babies transfer to therefore approval was required. The Centralised Service for NHS Permissions allows the use a single research governance application form but individual approval was required for each site. The NHS Health Research Authority propose to centralise this approval with national single sign off to be implemented in 2016

http://www.hra.nhs.uk/documents/2014/01/sponsorship-consultation-paper-final-1-0.pdf (accessed August 2015).

The Regulatory standards led to indirect improvements in clinical practice. Training clinical teams has increasing awareness of precision required for therapeutic drug concentration monitoring. Temperature monitoring of ward stock found that the neonatal unit's temperatures often exceeded recommended temperature resulting in the need for improved storage in NICU. Surprisingly, the storage of drugs on wards is not governed by the same standards as storage within the pharmacy department [318]. Training the clinical teams in sample precision has increased awareness of precision required for therapeutic drug level monitoring.

There are cost implications for mitigating risks effectively. Training 200 staff per site for 30 minutes is equivalent to 5.5 weeks full time clinical hours. The NIHR allocate service support costs for non-commercial research to NHS Trusts [319] for training clinical teams but the time and cost is underestimated. The cost of conducting a clinical trial increased following the Directive 2001/20/EC [320]. For example the Academy of Medical Science reported a disproportionate amount of time spent by staff preparing for inspections [235]. This proportionate model may reduce costs as Type A studies are less likely to be selected for inspection by the MHRA (daily rate per inspector £2583). The MHRA are moving towards reducing documentation by allowing electronic site files [234]. In this trial electronic site files were used for follow up sites.

A limitation of this proportionate approach is that it focuses on the risk of the IMP to categorise the study as A, B or C whereas the risk of data reliability in PK trials can be greater. Detecting precision errors is even more important for neonatal PK trial due to the small scale of drug volumes, blood samples and complex infusion lines so there is a risk of error. The consequences of unreliable data are considerable. Effective

monitoring for data reliability requires clinical knowledge to detect errors in sampling or drug administration and a detailed examination of source verification data. The MHRA risk stratification allows the Sponsor to authorise central monitoring for lower risk trials [232, 234, 296]. Unfortunately, central monitoring is often delegated to non-clinical personnel and focuses on governance checks. Drug administration and sampling can be mitigated by providing standard operating procedures specific to the clinical practice at the hospital and methods for cross checking data. The optimal model would include accredited sites for conducting clinical trial where there are standards for each stage of the process. This was the first PK trial at either recruiting site and detailed planning was undertaken in each area including the laboratory, wards and pharmacy. In addition to a thorough regulatory assessment, these insights have been shared with other research teams to prevent risks to neonatal PK trials.

This risk proportionate approach is an initiative of the MHRA in the UK therefore may not be applicable to trials with European partners. At present each EU member state has some discretion on how they interpret the EU Directive 2001/20/EC [235, 237]. The European Commission has passed a clinical trial Regulation that include some elements of proportionate risk management [321]. This will harmonise trial conduct and allow a more consistent approach to some of the regulations across Europe by binding each state rather than allowing each state to select clinical trial regulations in the directive. European collaboration is valuable when recruiting to neonatal trials as the sub-age groups result in small numbers of eligible patients especially for rare conditions such as GN sepsis. Approximately 24% of all EU clinical trials are performed in at least two Member States these larger trials involve approximately 67% of all subjects enrolled in a clinical trial [320].

This PK clinical trial model was published on the MHRA web site http://forums.mhra.gov.uk/forumdisplay.php?22-Risk-adaptive-approach then presented

to the European Medicines Agency towards standardising a risk proportionate approach throughout Europe [237]. The MHRA invited the research fellow to present this model to the EMA and FDA during the consultation process for the EU Regulation [321] to encourage a proportionate approach internationally. Many drugs are not authorised for children and a standardised approach to regulations throughout Europe is beneficial to achieve the objectives of the EU Paediatric Regulations to license drugs for children by the EMA [73]. This is highlighted by these PK and clinical outcome data.

Clinicians view by the regulatory process as a barrier and the complexity of the regulations results in misconceptions. Most of the regulatory adaptations implemented in this model were possible prior to the introduction of this risk proportionate approach but negotiation with the MHRA was required on a study by study basis. This caused delays and uncertainty and the advantage of this initiative is that adaptations are more transparent. Unfortunately, the process for applying for Type A may deter investigators as the MHRA require a detailed prospective risk assessment of the IMP with a plan to mitigate risks reviewed by the Sponsor, funders and other investigators [232]. Providing sufficient evidence of use in standard care for the population may be a barrier in the absence of a systematic review. A simpler approach would be for the MHRA to accept that the BNF C is viewed as sufficient evidence of use in standard care [173]. By publishing models for different types of trial design on the MRHA web site will provide useful templates and the recently published a GCP Guide will simplify the process for investigators [234].

### 5.4.1 Conclusion

Critically ill patients are a challenging vulnerable patient population and this proportionate approach considerably reduced the barriers to conducting a PK clinical trial. The model provides methods to mitigate potential risks to the patient and data reliability. Determining causality for pharmacovigilance reporting in critical illness can generate vast ‘nuisance data’ as causality cannot often be determined for the numerous adverse events. In most cases a blinded randomised controlled trial is required to determine causality due to the complexity of the illness, interventions and concomitant medication. It is evident from these data that a proportionate approach to monitoring adverse events is beneficial to reduce the burden to the clinical team. Events more likely to be reactions and provide new safety knowledge are not missed by the burden of over reporting un-related events.

Even though this drug is prescribed off label to a vulnerable population the MHRA awarded a Clinical Trial Authorisation for a Type A trial as comparable to standard care. The need for a more proportionate approach is highlighted by this study of neonates as many medicines are prescribed as part of standard care off label in paediatrics. PK trials of medicines used in standard care require an adaptation for a lower risk model compared to early phase trials of healthy volunteers that have informed many of the regulatory standards. The proposed framework provides a more transparent and proportionate method appropriate to the complexity of children in intensive care. This model provides a more proportionate application of the regulations that are specific to the investigational medicinal product and the population rather than a ‘one size fits all’ approach at the same time as minimising potential risks to the patient or data reliability.

# Chapter 6 Final Discussion

## 6.1 Implications for Clinical Practice

Ciprofloxacin is an option for the treatment of suspected sepsis in neonates and young infants. Optimising existing therapy to treat Gram-negative bacteraemia is essential due to the severity of illness, rapidly increasing resistance and lack of new antimicrobials in development [6]. The burden of Gram-negative bacteraemia is evident in this neonatal unit as when Gram-negative bacteraemia was confirmed mortality increased to 44% compared to approximately 20% for those <28 weeks PMA or 8% for those 28-34 weeks PMA. Sub-optimal antimicrobial drug exposure also increases the generation of resistance [322]. This is evident when medicines are administered off label by the wide variation in regimen, which further compounds PK variability and increases resistance. Ciprofloxacin regimens internationally range between 5 to 60 mg/kg/day [184]. The preliminary PK-PD data in this thesis contribute towards optimising the regimens and authorising ciprofloxacin for neonates and young infants.

The strength of this popPK clinical trial is that the data were acquired in a real world setting where the effect of critical illness and rapid changes in organ development from extreme prematurity to young infants. Of the 64 neonates and young infants recruited a minimum of seven babies were stratified to each four-week sub-age group. Extreme variation is evident between subjects even within each individual sub-age group. For neonates 28-31 weeks PMA the $AUC_{0-24}$ (SD) ranged between 65 – 291 mg*h/L (60). This extensive variability illustrates the limitations imposed by a fixed dosing strategy and the potential benefit of individualised therapy. However, there are significant practical implications to personalising therapy. Covariate analysis found that gestational age, postnatal age, current weight, serum creatinine and inotrope administration had a significant impact on ciprofloxacin PK parameters. A pragmatic

approach is to adjust ciprofloxacin regimen using these covariates that have an impact on clearance associated with either maturation or altered renal function during critical illness. As the combined effect of post-natal age and post menstrual age at birth affected clearance, different regimens may be required for neonates with the same post-natal age depending on how premature they were at birth. More frequent dose intervals are indicated for neonates >34 or 36 weeks PMA from 12 to 8 hourly than are currently recommended in the BNF C [173]. These PK data found that neonates >34 weeks PMA may be undertreated with 20mg/kg/day as only 66% achieved the conventional AUC/MIC 125 target estimated by Monte Carlo simulation. Individual AUC data was available for each patient and 14% of those treated did not achieve the AUC/MIC ratio >125. The optimal dose of ciprofloxacin is dependent on establishing the optimal predictors for therapeutic success specific to neonates and infants which is currently unknown.

Pharmacodynamic studies specific to neonates and young infants are uncommon. The conventional target of an AUC/MIC 125 for optimising therapeutic success is based on adult outcomes and regimen [162]. More recent paediatric and adult data supports a higher ratio particularly for severe infection [115, 176]. Zelenetski et al in 2010 [176] found a ratio of >250 was required to achieve therapeutic success reliably. Fewer than half of our trial participants (42%) achieved this target when administered 20 – 30 mg/kg/day (Chapter 2). Over a six year period the incidence of this condition was insufficient to generate statistically significant findings in a study relating susceptibility to ciprofloxacin outcomes. Vast numbers of babies would be required to power the necessary efficacy and safety studies, which is challenging. In this large tertiary neonatal unit the incidence of Gram-negative bacteraemia was only 1.3%. The data described in Chapter 4 indicates that the ‘lower’ dose 10mg/kg/day was only sufficient for therapeutic success for isolates with an MIC <0.035mg/L, considerably lower than the non-species specific clinical breakpoint. Clinically this is significant as

44% of isolates had an MIC greater than this yet are currently classified as susceptible according to the EUCAST clinical breakpoint. Therefore these neonates may have been undertreated for those prescribed ciprofloxacin. Lipman's PK data for infants >3 months of age found 20mg/kg/day was only sufficient for organisms with an MIC of <0.24 mg/L [115]. This outcome study suggest that in neonates given ciprofloxacin at a dose of 5 mg/kg 12hourly for GNS, there is a marked difference in clinical outcome depending on the ciprofloxacin MICs, even though this was within the susceptible breakpoint range. This was not statistically significant but indicates that either a lower clinical breakpoint or higher dose may be required for bacterial inhibition if a higher dose can be tolerated without significant adverse reactions. Further analysis of the PK data is planned to estimate the dose and interval to achieve an AUC/MIC >250.

In addition to the MIC the pathogenicity of the bacteria is likely to determine the outcome. The rate of cure for *Enterobacter* spp. was much higher (89%) than *E.coli* (44%) for a similar MIC. In contrast, the median MIC for *Pseudomonas* spp. was much higher than *E.coli* with a similar outcome to *E.coli.* These outcome data indicate that the optimal regimens may be dictated by the type of bacteria, the MIC and age. Administering higher doses for more pathogenic and less susceptible organisms may improve clinical outcome. These data are not definitive but strengthen the need for PK-PD data that is specific to each age group and clinical context of severe illness. Ideally with AUIC data that compares the outcome with both the MIC and PK data for the same patient.

Variability in PK parameters exists even between age sub-groups. The PK clinical trial (Chapter 2) found clearance increased three-fold within these age groups (range 0.11 to 0.35 L/kg/h). These limited clinical outcome data (Chapter 4) suggests that the more premature babies tended to have an improved outcome. This was unexpected as mortality is higher with extreme prematurity and whilst recognising these data were not statistically significant it is worth consideration. It may be due to a higher AUC

possibly due to relatively low clearance associated with their immature renal function. Other factors such as disease progression confound this assessment highlighting the challenge and complexity of outcome assessments in critical illness. Although the higher AUC may be beneficial caution is required as this may result in toxicity. There is a wide range in half-life (range 2.8 to 33.8 hours). Gentamicin has extended dose intervals up to 36 hours in neonates and regimens are stratified according to sub age groups of <29 , 29-35 and >35 weeks PMA. Dosing intervals may need to be specific to the individual as targeting the mean or median AUC even for a sub-age group will inevitably undertreat those above the targeted range.

Variability within an individual patient over the period of sepsis and inflammatory response requires further analysis as rapid changes in organ function and weight are extreme in neonates and infants. As dosages are based on weight in paediatrics the pre septic weight is often used for calculations. This method may be appropriate for ciprofloxacin as concentrations are considered to be affected less by the fluid shifts caused by inflammation and fluid resuscitation than hydrophilic drugs. The effect of critical illness on PK parameters has been described in detail but there is little published data on the later stage of sepsis as inflammation resolves. Frequent adjustment may be required over the sepsis period for example as inflammation resolves the drug may re-enter the central compartment resulting in higher concentrations. Further individual analysis is planned to estimate the change in concentrations for the paired early and last day samples. Some variability in concentrations may be due to imprecise measurement. The risk to precision is greater in neonatal PK studies due to the minute drug volumes, minute samples and complex administration lines. Close agreement between scavenged and PK timed samples was achieved. This suggests data reliability is achievable with rigorous planning, training of the vast number of clinical staff, bedside tools and standard operating procedures specific to the site.

Resistance of Gram-negative bacteria are reported to be increasing rapidly throughout Europe and is of increasing concern due to the lack of new antimicrobials [6]. Over the six year retrospective study (Chapter 4) the incidence of confirmed Gram-negative bacteraemia was too low to determine whether annual changes in susceptibility occurred. Surveillance data of surface swabs over a thirteen year period found the incidence of ciprofloxacin resistance was no greater than for gentamicin although ciprofloxacin is prescribed less frequently. Sub-optimal treatment with ciprofloxacin increases the risk of resistance due to selection pressures as some antibacterial concentrations allow bacterial mutants to thrive [48]. Roberts et al review of antibiotic resistance concluded the highest tolerated dosing regimen must target the most resistant populations in an effort to prevent the emergence of resistant organisms due to selection pressures [48]. Increasing resistance of Gram-negative bacteria may be due to sub-optimal antibiotics regimens rather than related to usage over time. The mutant preventive concentration inhibits the least susceptible mutant within the wild type [48, 164, 166, 303]. The clinical relevance is unclear as further safety data is required for higher doses.

These neonatal data in Chapter 4 found higher MIC were associated with treatment failure (RR 2.5). This is consistent with recent reports of higher MICs in the susceptible range resulting in a recommendation to revise clinical breakpoints [67, 163]. These data were not statistically significant but indicate that the regimen at the time would only have treated bacteria with MIC less than the clinical breakpoint derived from adult outcomes. Data from the clinical trial reported in Chapter 2 shows that some neonates restarted ciprofloxacin on as many as three occasions during their admission. Retreatment may have been due to a new episode of presumed sepsis however this is less likely as this reoccurred within short intervals. Children are approximately 20% of the population and many antimicrobials are prescribed off label resulting in the risk of sub-optimal dosing allowing mutant bacteria to. One hypothesis is that this may be

fuelling resistance. Further safety data is required to determine the maximum dose tolerated.

In these critically ill babies adverse events not related to microbiology confounded causality assessment. The observational study in PICU and review of neonatal discharge letters (Chapter 5) has shown a vast number of adverse events are simultaneously associated with the clinical condition, clinical intervention and co-medication. Adverse clinical events such as abnormal biochemistry values are the nature of critical illness. In this trial safety data was purposefully reported rather than analysed as definitive causality assessment would require a blinded RCT. In the absence of a blinded RCT there is a risk of attributing events associated with critical illness to the administration of the investigational medicinal product. There were no suspected unexpected serious adverse reactions (SUSAR) and no clear, novel safety signal or evidence of unanticipated clinical events, acute articular problems, excess phlebitis or clinically significant derangements in laboratory parameters attributed to ciprofloxacin.

The main safety concern historically is the potential risk of arthralgia / tendonitis that may be underreported in this trial as there is no validated method for assessment in a non-weight bearing non-vocal population. These musculoskeletal events are potentially reversible [190]. Arguably the benefits of ciprofloxacin as an effective treatment for Gram-negative bacteraemia with high mortality out weights joint concerns that resolve with management. Seizures have been associated with higher Cmax in children. In the trial reported in Chapter 2, ciprofloxacin was found to penetrate the CSF by 0.33 of the serum concentration which may enhance treatment of meningitis but may result in neurotoxicity. Seizures were reported in this population even prior to ciprofloxacin administration and no temporal causality was found post administration. A pre-clinical study of high dose regimen in mice pleads for careful watching of cardiorespiratory and motor development in neonates [266]. The

Liverpool Archive of MRI in Babies (LAMB study) is a registry including scans of the hip, liver and brain of healthy neonates and patients some of whom were administered ciprofloxacin that will provide short term safety outcome data. Further long term safety outcomes are required. Potential risks of adverse events must be balanced against the benefits of improving the clinical outcome and adverse reactions to alternative antimicrobials (such as gentamicin). Short and long term safety data with a dose ranging study and blinded RCT is required to determine the maximum dose tolerated without significant effects.

### 6.2 Limitations

There are challenges to obtaining statistically significant PK-PD data especially when stratifying to sub-age groups. Vast numbers of babies would be required to power the necessary safety or efficacy studies due to the confounding effects of critical illness. The incidence of confirmed Gram-negative bacteraemia is rare and despite approximately 1200 admissions to the neonatal unit per annum these data are not statistically significant. Clinical outcome of Gram-negative bacteraemia for those in the trial cannot be determined as most participants were treated for suspected rather than confirmed bacteraemia and few had Gram-negative blood cultures. Ciprofloxacin is infrequently administered at either of the recruiting sites and no infants >48 weeks PMA received ciprofloxacin. Also, these data excluded neonates treated within five days of birth. Ciprofloxacin is mainly a second line agent therefore clinical outcomes may be influenced by first line therapy or co-administration of other antibiotics for example in these centres amoxicillin/ clavulanic acid or illness progression. Clinical breakpoints are informed by several studies and ideally thousands of MICs collected internationally. These outcome data illustrate the challenges of setting paediatric clinical breakpoints due to the limitations of the small number of neonates with confirmed bacteraemia treated with Ciprofloxacin. Even over a six year period in this tertiary neonatal unit despite retrieving most isolates this

resulted in as few as 88 MIC's. Only 33 of the patients were treated with ciprofloxacin therefore these clinical outcome data were insufficient for a conclusive analysis but the trend is consistent. This is insufficient to inform a paediatric clinical breakpoints as EUCAST often include PD data several thousand MIC's and collected internationally. A further challenge is that paediatric breakpoints would vary depending on each developmental stage and need to be stratified according to each sub-age group with PK and PD data specific to each age. There would still be variability with these sub-populations due to critical illness. An individualised regimen informed by rapid methods of detecting organisms and MIC's maybe a more pragmatic approach.

Definitive safety and outcome data require an RCT design due to the complexity of critical illness. Nevertheless, descriptive data can provide some support to prescribers. The definition of cure was pragmatic as at present there is no consensus on standardised outcome measures specific to neonatal sepsis [27, 29]. The PopPK model combines sample data from both the first and last days of treatment. Therefore further analysis is planned to assess the potential impact on PK Parameters over the early and late stage of sepsis on PK. This data may inform individualised therapy.

### 6.3 Further Recommendations

This research further supports the need for individualised therapy supported by real time therapeutic drug monitoring. This is challenging due to limitations on blood sampling and because the organism and the MIC is not usually known at the start of treatment. Real time therapeutic drug monitoring would require rapid throughput of ciprofloxacin assays and tests that identify bacteria. New methods for rapid identification including MALDI-TOF-MS a mass spectrometer and PCR assays with real time therapeutic drug monitoring may facilitate this. There are practical limitations of PCR due to cost and availability for clinical laboratories, assays are limited at present and universal detection systems for clinical diagnostics would be optimal

[323]. Banerjee et al RCT found the time from Blood Culture Bottle Gram stain to microorganism identification was shorter rapid multiplex PCR (rmPCR) (1.3 hours) vs conventional methods (22.3 hours) ($P < .001$). Using a template for antimicrobial stewardship they demonstrated reduced treatment of contaminants and use of broad-spectrum antimicrobials and antimicrobial de-escalation [324]. Whilst this increased the cost and complexity of patient care it may be balance against the potential for shorter inpatient stay and improved outcome. There is a risk that this method may detect low level concentrations of pathogen microbial DNA after successful treatment as the assay may detect viable and non-viable organisms [325] [326]. A false positive may be detected from exogenous DNA contamination or false negative if the sample is too small [327]. Equally, MALDI-TOF-MS is limited to specific species. In addition there are newer methods for rapid phenotypic susceptibility testing (sample to MIC). There is a need to determine resistant genes rapidly and Accelerate Diagnostics Inc www.acceleratediagnostics.com/products/asm2015symposium/ (Arizona, US) produce a system that identifies an MIC within 5 hours of a culture and includes the main Gram-negative organisms in found in these studies and ciprofloxacin [328].

There are limitations to the PK data produced in this highly variable population influenced by both critical illness and rapid development changes as illustrated by the wide range of AUC even within sub-age groups. Drug levels vary between doses. There is a need for further neonatal PK studies to guide prescribing of antimicrobials in particular the initial dosing for that population however it is evident from these data that the wide range of AUC even within a sub-age group requires real time characterisation, MIC and PK based on dose adjustment.

A unique opportunity exists in this population as the frequent clinical samples for blood gas analysis, biochemistry and haematology provide an opportunity to scavenge samples for PK analysis including priori data. Data reliability is dependent on precision by the wider team when drugs are administered day and night. Clinical teams and

parents were engaged by conveying the importance of the research to future neonates and developing a research culture sensitive to such a stressful situation. Establishing accredited PK-ready clinical wards and laboratories would enhance data reliability with standards for conducting PK trials, training clinical teams and recording administration data on drug charts. Neonatal PK studies are rarely repeated; these data can influence prescribing practice internationally therefore data reliability is ethically essential.

The utility of interpreting PK data is limited in the absence of PD and safety data ideally AUIC comparing the dose and concentration in a patient with their outcome. Further population PK/PD studies will support the development of clinical breakpoints for children. Knowledge of the drug concentration achieved does not indicate efficacy without clinical outcome data specific to the PK for that patient. This clinical outcome study has illustrated that without PK data the outcome of the patient and the dose administered can equally indicate efficacy without the PK concentration. However, PK concentrations are useful to determine covariates allowing an estimate of the optimal initial dose and a baseline to monitor safety. Neonatal and paediatric clinical breakpoints informed by pharmacokinetic, safety, microbiological and clinical outcome data are required. Clinical breakpoints estimate the susceptibility of an organism by estimating the MIC that will be treated effectively by a specific dose whereas the ECOFF is a laboratory measure of susceptibility. The true value of an MIC is as a measuring tool that generates values to which other parameters such as PD endpoints and clinical outcomes can be reliably compared [153]. The paediatric outcome and drug regimen are vastly different to adults. At present clinical breakpoints are solely derived from adult data as referenced in the rationale documents [161]. This study emphasises the need for PK/PD data for antimicrobials in children. A modified breakpoint model may be required for each sub-age group of children. This is challenging as seen in the PK clinical trial there are as many as seven

sub-age groups just for neonates and the EMA recommend a further four for children [79, 80]. Clinical Breakpoints even for adults are often informed by relatively few PK papers for example the ciprofloxacin rationale document by EUCAST [56] cites only seven PK papers all dated before the year 2000 yet influences prescribing practices globally. Undoubtedly, there are challenges to obtaining sufficient neonatal PK/PD data evident in this study that have resulted in many drugs being prescribed off label. However, newer methods such as population PK and the additional incentives provided by the Paediatric Regulation may be the foundation for developing a framework for future paediatric breakpoints. The need to optimise therapy is not only for efficacy but also to reduce resistance. Sub-optimal therapy may be increasing resistance correlated to the over use of antimicrobials as opposed to the over use itself being the cause. Increasing evidence of the burden of resistance International collaboration of neonatal units is required that may be facilitated by recent initiatives including the European Union Paediatric Regulations in 2006 and the EU Clinical Trials Regulation implemented in 2015 [321] and contribute to an evaluation of a paediatric breakpoint model.

Practical solutions to obtaining reliable neonatal PKPD data were developed during the research. This included designing a model to reduce regulatory barriers by the Risk Proportionate Approach for PK clinical trials in collaboration with the MHRA. In addition to less invasive methods for sampling PK blood concentrations and DNA by scavenging from of clinical samples. As this was the first PK clinical trial in either recruiting site a PK clinical trial culture was established within the neonatal and paediatric intensive care units for future studies. This PD cohort study of clinical outcome provides a non-invasive framework for systematic PD surveillance. In the absence of PK clinical trial data this model may provide an interim framework to monitor the outcome of ore severe infection such as bacteraemia and meningitis comparing the MIC and clinical outcome.

## 6.4 Conclusion

PK-PD for neonates and young infants is challenging. This is evident by the fact that so many antimicrobials are unauthorised and prescribed off label, as a result of which there is a lack of data available to contribute to clinical breakpoints. Gram-negative bacteraemia in this vulnerable population has severe consequences. Despite advances in intensive care adverse outcomes of sepsis are likely to increase due to the rapid development of resistance to antimicrobials. Optimising therapy aims to improve outcome at the same time as minimising resistance. These preliminary data contribute to optimising ciprofloxacin therapy for this population. The incidence is relatively rare and collaboration of national and international neonatal and paediatric units is required to obtain statistically significant data representative of the effects of ontogeny on each sub-age group. The popPK model reflects the rapid changes during development and impact of illness. A bespoke trial design mitigated the risks to the subjects and the data whilst adhering to ethical and regulatory standards. Methods developed in this research may contribute to a PK-PD framework for future research. Optimising PK-PD data for antimicrobials that target Gram-negative organism is essential with the rapidly increasing threat of resistant organisms.

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**APPENDIX I**

# Clinical Trial Protocol

TREAT INFECTIONS IN NEONATES

## To evaluate the pharmacokinetics, tolerability and short-term safety of ciprofloxacin in neonates with suspected (or proven) Gram Negative infection

## Phase I, open-label pilot PK Study -TINN Treat Infections in Neonates Program

**Version:** 1

**Sponsor Reference:** LW0852

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Synopsis

Synopsis: ‘Treat Infection in Neonates’ (TINN) Ciprofloxacin Pharmacokinetic (PK) Study

| | |
|---|---|
| Type of study | Pilot Population PK study - Clinical Trial |
| Study Design | Phase I, open-label study to evaluate the pharmacokinetics, tolerability and short-term safety of ciprofloxacin in neonates with suspected (or proven) Gram Negative infection. |
| Type of control | Nil |
| Location | Liverpool Women’s NHS Foundation Trust (Neonates)<br>Alder Hey Children's NHS Foundation Trust, Liverpool UK |
| Test products | Ciprofloxacin |
| Dosage regimen | 10 mg / kg / dose, 12 hourly (adjusted if indicated by interim analysis) |
| Route of administration | Intravenous, 30 - 60 minutes infusion |
| Objective(s) of the study | To evaluate the multiple-dose pharmacokinetics of ciprofloxacin in neonates and young infants (24 – 52 weeks postmenstrual age) with suspected or proven Gram Negative infection.<br>To evaluate the tolerability and describe short-term safety of ciprofloxacin in neonates and young infants with suspected (or proven) Gram Negative infection.<br>To describe the clinical outcomes of neonates treated with ciprofloxacin |
| Sample size | 50 patients between 24 -52 weeks postmenstrual age |
| M/F | not predetermined |
| Sample Size<br>(Pro rata to the ICH EMEA age-groups for paediatric studies) | 50 neonates stratified according to postmenstrual age (premature or not) to represent ages between 24 -52 weeks with 5- 8 patients in eachba 4 /5 week period.<br>The target recruitment is 5-8 patients to represent each 4/5 week period in the range 24-27, 28-31, 32-35, 36-39, 40-43, 44-47 and 48-52 weeks postmenstrual age. |

| | |
|---|---|
| | In order to attain this number of samples on day 5- 7, we will need to recruit more participants than this as some participants will die, others will move to other centres and some parents will decline repeated sampling. |
| Study Interventions | Sparse blood samples (n=2 or 3 depending on weight) will be drawn on day 1 and day 5- 7 (or last day of treatment if the course is completed before day 7).<br>Monitoring of adverse events<br>DNA for pharmacogenetics (scavenged clinical samples or buccal)<br>CSF (if required clinically)<br>Faeces |
| Duration of ciprofloxacin treatment | At least 5 days |
| Duration of follow up | 3 days after completion of ciprofloxacin treatment (plus stool sample 4 -6weeks after completion of ciprofloxacin) |
| Inclusion criteria | Receiving ciprofloxacin following clinical decision by attending physician |
| Exclusion criteria | Likely not to survive 48 hours in the judgement of attending physician |
| Endpoints | |
| Primary | Ciprofloxacin plasma concentration and population pharmacokinetic (PK) parameters [maximum concentration, clearance, area under the curve (0-tau)], their relationship with selected covariates their interindividual variability (CV%). Covariate analysis will include postmenstrual age, gestational age, postnatal age, weight, and serum creatinine |
| Secondary | 1. PK variables, including apparent volume of distribution and half life.<br>2. Withdrawal due to lack of tolerability<br>3. Adverse events (AEs) and serious adverse events (SAEs). |

| | |
|---|---|
| | 4. Outcome of treatment episodes (clinical and microbiological) |
| Power calculation | This is not a hypothesis-testing study. The pharmacokinetic (PK) data generated from this study will assist in dose selection for use in neonates and infants. At this stage there is no data for this medicine in this age group. Accordingly, sample size calculations are not possible for this pilot study. Sample size and number in each age-range have been based on the experience of the PK scientists involved in the study. |
| Options | |
| Recruitment issues | If recruitment is poor, other sites will be approached. |
| Interim analyses | Blood levels will be monitored after every 10 patients recruited and interim pharmacokinetic analyses will be conducted, following which adjustments may be made to optimise the dose based on PK PD modelling. |
| Stopping rules | None anticipated |
| Statistical methods | |
| Primary analysis | Population pharmacokinetic analyses will be performed and the effect of covariates. This preliminary POP-PK model (PK parameters and their variability estimates in the 50 neonates and infants) will be used for simulations to determine the optimal dosing regimen in this population. The optimal dose will be defined based on the pharmacokinetic-pharmacodynamic break points extrapolated from adult patients: $AUC_{0\text{-}24}$/MIC (AUIC) >100 for gram-negative pathogens. The optimal dose will be evaluated in further clinical studies. |
| Secondary analyses | PK: to investigate the potential effects of other covariates.<br>Tolerability: proportion of participants who withdraw due to intolerance of ciprofloxacin as judged by attending clinicians<br>Safety: descriptive statistics of adverse events<br>Outcome of treatment episodes: proportion of participants who have recover within 3 days of stopping ciprofloxacin.<br>Pharmacogenetic analyses will focus on ciprofloxacin transporters (OAT3, BCRP). |

Time and Events Schedule

| Action | Following admission to the neonatal unit | At time judged suitable by clinical staff | Clinicall suspicion of sepsis | Clinical decision to start cipro-floxacin | First dose of cipro-floxacin | Sampling | | Final dose of cipro-floxacin | 3 days after completion of cipro-floxacin | 4 – 6 weeks after completion of antibiotics |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Day 1 | Day 5-7 | | | |
| Parents or legally appointed representative told about study | X | | | | | | | | | |
| Formal discussion with parents or legally appointed representative | | X | | X* | | | | | | |
| Consent | | X | | X* | | | | | | |
| Blood culture as per clinical practice | | | X | | | | | | | |
| Gram Negative (suspected or proven) | | | | X | | | | | | |
| Enrolment (when eligible) | | | | X | | | | | | |
| Ciprofloxacin administered | | | | | X | | | | | |
| Baseline safety blood sample# | | | | | X | | | | | |
| Repeat safety blood sample# | | | | | | | | X | X | |
| PK blood sample and pharmacogenetics | | | | | | X | X | | | |
| CSF sample if required clinically and if consented | | | | X | | | | | | |
| Safety data collection | | | | X | X | | | X | | |
| Safety evaluation | | | | | | | | X | X | |
| Faeces sample | | X | | | | | | | | X |
| DNA sample – if consented | | X | | | | | | | | |
| DNA sample from blood required for clinical care if consent obtained | | | | X | | | | | | |
| Test-of-cure | | | | | | | | | X | |

* Formal discussion about the study and consent will be undertaken before clinical suspicion of sepsis when possible but in some cases consent will be requested at the time the baby is assessed to be septic.

# Safety blood samples will be clinically indicated sampling episodes that may be slightly before or after the start or finish of ciprofloxaci

Abbreviations

| | |
|---|---|
| AUC | Area Under the Curve |
| $AUC_{24h}$ | Area Under the Curve over 24h |
| CRP | C Reactive Protein |
| CSF | Cerebrospinal fluid |
| MHRA | Medicines and Health Care Products Regulatory Agency |
| MIC | Minimum Inhibitory Concentration |
| PK | Pharmacokinetic |
| VLBW | Very Low Birth Weight |

Notes: The term 'parent ' includes those with parental responsibility throughout this document.

The term ‘Sponsor’ refers to which ever co-sponsor has responsibility for the issue under discussion, as defined in the written agreement between the co-sponsors.

Introduction

We propose to conduct a pharmacokinetic study in preterm and term neonates and infants up to 3 months post menstrual age with suspected (or proven) systemic gram negative infection, collecting pharmacokinetic data using sparse sampling techniques. Appropriate pharmacokinetic parameters will be assessed (e.g., AUC, but also apparent clearance, $T_{1/2}$, apparent volume of distribution, $C_{max}$ and others as appropriate). Pharmacokinetic characteristics following repeated dose will be evaluated and compared to those of the first dose.

The EU Paediatric Regulation [1] introduced in 2006 aims to ensure that a medicinal product for use in children undergoes extensive studies to ensure that it is safe of high quality and for use in the target population. Many products used in the paediatric population have not been authorised for such use. In particular, there is often inadequate dosage information which leads to increased risks of adverse reactions including death and ineffective treatment through under dosing.

The EU Paediatric Regulation recognised the failure of market forces to provide appropriate medicines for children, particularly neonates. In order to address this failure the Regulation introduced a Paediatric Use Marketing Authorisation (PUMA). To encourage PUMA applications the Commission also established funding for collaborative research projects under Framework Programme 7. Projects were expected to address the needs contained with the European Medicines Agency (EMA) priority list of off-patent medicines current at the time [2]. One project to receive this funding is the Treat Infections in NeoNates (TINN) collaboration led by Prof. E. Jacqz-Aigrain in Paris. TINN will assess two antimicrobials, ciprofloxacin and fluconazole. This protocol relates to one aspect of the research programme in ciprofloxacin.

The WHO Expert Subcommittee considering Essential Medicines for Children concluded that *"sufficient evidence is available to support the use of ciprofloxacin as a second-line treatment for specific, severe infections in paediatric patients.* It has been included as the only fluoroquinolone on the list of Essential Medicines for Children prepared by WHO [3]. Indications include concern about antibiotic resistance in particular settings, or when other classes of antimicrobials (e.g. aminogylcosides) are contraindicated due to toxicity.

Ciprofloxacin is not a common choice of antibiotic in neonates. Nevertheless, it is a useful option in some circumstances. We have conducted a systematic review which found reports of ciprofloxacin use in several settings in Europe and Asia (Kaguelidou, personal communication). The TINN consortium has conducted a web-based survey of practice in Europe. 193 units responded including units from 20 countries between Uzbekistan and Portugal. 50 of 193 units (26%) use ciprofloxacin at least occasionally (Pandolfini, personal communication).

Among these units, the indications for using ciprofloxacin were:

| **Indication** | **Number of units** | **Percentage of units using this indication for ciprofloxacin** |
|---|---|---|
| First line therapy neonatal sepsis | 1 | 2% |
| Severe neonatal sepsis | 3 | 6% |
| Neonatal sepsis only when CNS documented infection (meningitis, meningoencephalitis, ventriculitis, cerebral abscesses…) | 13 | 26% |
| Neonatal sepsis resulting from a suspected multi-drug resistant strain infection | 15 | 30% |
| Neonatal sepsis resistant to first line empirical antibiotic therapy (other than ciprofloxacin) | 7 | 14% |
| Severe neonatal sepsis resistant to first line empirical antibiotic therapy (other than ciprofloxacin) | 17 | 34% |
| Culture-proven sepsis due to multi-drug resistant organisms but sensitive to ciprofloxacin | 41 | 82% |
| NB Units could give more than one indication | | |

Although this survey relied on self-report it does show that some units use ciprofloxacin in neonates.

Pharmacokinetic data of ciprofloxacin in neonates are extremely limited (see below). In the TINN survey there was significant variation in dosage regimen.

| Total dose /kg / day | Number of units | Percentage of units |
|---|---|---|
| ≤ 10 | 11 | (22%) |
| | | |
| 11-20 | 20 | (40%) |
| 21-30 | 9 | (18%) |
| >30 | 1 | (2%) |
| (missing = 9) | | |

This justifies more detailed study of this medication in this population. The literature suggests that the efficacy of ciprofloxacin in neonates is at least comparable to other antibiotics used in cases of suspected (or proven) Gram Negative neonatal sepsis. The advantages of more information about ciprofloxacin include more robust dosage information for those units that already use it. This includes many units in resource-constrained settings. Units that do not use it due to uncertainty about dosage may find it more useful if dosage information is available. In units with resistance to other antibiotics it may be possible to use ciprofloxacin. Disadvantages to more widespread use of ciprofloxacin might be increased selective pressure for resistance, but this is not unique to ciprofloxacin. There is a risk that ciprofloxacin could cause adverse reactions. The potential reaction that might concern clinicians the most is arthropathy or tendinopathy. As noted in section 5.3 there is no specific evidence that these problems are caused by ciprofloxacin in neonates. The TINN registry will collect data about safety in babies exposed to ciprofloxacin, this will then be held in Paris. This data will allow clinicians to weigh the potential risks against the potential benefits.

It is necessary to extend the evidence base used to support prescribing of this medication in neonates. Pharmacokinetic data from adult, adolescent, and older children cannot readily be extrapolated to the infant patient population. Varying levels of protein binding, metabolic pathway maturity and renal function in infants can have profound implications on drug disposition (ICH Topic E11) [4-5]. Extant literature provides PK data for infants aged 3 months and older (Lipman et al. [6]). This study will examine ciprofloxacin PK in infants aged less than 3 months and in neonates of all gestational ages.

The TINN collaboration has submitted a Paediatric Investigation Plan to EMA. We have received comments from the Paediatric Committee (PDCO) at the day 60 stage of the PIP process. The PDCO have agreed in principle that further data is not needed for children aged 3 months and older so that further work will examine infants aged less than 3 months and neonates. The ciprofloxacin PIP will include some preclinical studies and the development of a formulation appropriate for neonates. The clinical development programme will describe PK and

safety. Efficacy will not be studied because the efficacy of ciprofloxacin can be assumed in this age group based on its effects in older age-groups [7].

This study will gather the data required for an initial PK analysis in this age-group. Data from this study will be combined with pre-existing data about physiology in this age range to model concentrations of ciprofloxacin in neonates, that is, a physiologically based PK (PBPK) model will be developed. In turn this will be combined with information about the concentrations of ciprofloxacin required to treat Gram Negative infections. This will lead to an optimised dosage recommendation in this age group. The PK study will be undertaken in the UK. PDCO recommended that recruitment is limited to one neonatal unit and one paediatric unit, but this may be reviewed to achieve targeted recruitment. The optimized dosage recommendation will be evaluated in further clinical, specifically PK sampling among patients recruited to a European Neonatal and young infant ciprofloxacin TINN registry. Therefore the PK study will establish a framework for ongoing data collection in the Registry.

The separate **TINN Ciprofloxacin European Registry** study will assess safety and the outcome of treatment episodes involving ciprofloxacin. This PK study will also provide some short term safety information. Participants in this PK study will also be eligible for the registry: the registry will involve separate consent. The registry will also include further opportunistic samples to extend the PK models and will include a pharmacogenetic module. Participants in the PK study will be eligible for the pharmacogenetic study.

Ciprofloxacin has been used as second line treatment for neonatal sepsis on the Neonatal Unit at Liverpool Women's Hospital for twenty years. It is used at Alder Hey Children's Hospital for hospital acquired infections. This setting provides an opportunity to study this agent on a population who are receiving it according to physician preference.

*5.1 Neonatal sepsis*

Definition -**Neonatal sepsis is defined as "a clinical syndrome characterized by systemic signs of infection and accompanied by bacteremia in the first month of life" [8].**

Neonatal sepsis is generally classified as "early onset" and "late onset". Early onset sepsis is likely to reflect infection with organisms acquired before, or during birth. Late onset sepsis occurs more than 72 hours after birth and is likely to reflect nosocomial infection. The incidence of neonatal sepsis is inversely proportional to gestational age and birth weight. Very low birth-

weight infants (VLBW < 1500g) represent 5% of all births. Among VLBW infants culture-proven early onset sepsis is seen in 2% of infants and late-onset sepsis in 25% of infants [9-10]. All cause mortality is approximately 25% in early-onset sepsis and 18% in late-onset sepsis in VLBW infants [9, 11].

**Prevalence-** Gram-negative neonatal sepsis is a significant problem in neonates, especially VLBW infants [12-13]. In developed countries, although there is a wide variation between units regarding the type of germs responsible of late infections in VLBW infants, Gram-negative bacteria account for approximately half of the early onset and one third of the late onset neonatal infections [10-11] whereas in developing countries Gram-negative organisms remain the major cause of neonatal sepsis [11] [14]. Currently, the most common Gram-negative organisms isolated in cases of neonatal sepsis are E.coli, Klebsiella and Pseudomonas species [11-13]. The overall mortality due to Gram-negative sepsis varies from 19 to 52% depending on the infecting organism (Pseudomonas aeruginosa infections are generally more lethal) and host factors (gestational age and birth weight)[11, 13, 15].

**Morbidity & Mortality** - The overall mortality due to Gram-negative sepsis varies from 19 to 52% depending on the infecting organism (Pseudomonas aeruginosa infections are generally more lethal) and host factors (gestational age and birth weight) [13]. Mortality due to Gram-negative infections is significantly higher than that of Gram-positive infections at all ages of sepsis onset [13, 16-17]. Fulminant late-onset sepsis (i.e. sepsis that is lethal within 48 hours) is more likely to be caused by Gram-negative organisms [17]. Hospital acquired infections from Gram-negative bacilli may be increasing [17-18].

Important aspects of brain development are ongoing during the neonatal period, particularly among premature and VLBW infants. There is a significant risk of long-term neurodevelopmental sequelae among survivors of Gram-negative infections, particularly when the disease is complicated by meningitis [15]. Brain development can be disrupted by ill-health during the neonatal period. Developmental delay and disability is increased among children who were unwell during the neonatal period. It has been suggested that recurrent postnatal infections can influence early biomarkers of brain development [14].

The antibiotic regimen used to treat neonatal sepsis varies considerably between neonatal units and between countries. This variation is due to the pattern of resistance to antibiotics observed in different units. In some settings multiply resistant bacteria are found. In these settings it can be difficult to select an adequate antibiotic. When faced with difficult choices about antibiotic usage, some units have elected to use ciprofloxacin. The literature provides a strong prime facie case that ciprofloxacin has efficacy in neonates with suspected (or proven) Gram negative infection.

**Neonatal/Infant Systematic Review - Ciprofloxacin -**The published experience of ciprofloxacin in other neonatal centers has been summarized by Kaguelidou et al. following a systematic review (submitted for publication). They found 5 cohort studies. Measures of treatment outcome were available in only 2 cohort studies**.** The first [19] , reported a clinical response rate of 64% among 86 ciprofloxacin-treated neonates compared to 27% among 344 controls. The second study [20] reported a global survival rate of 91% for the ciprofloxacin group (n=116) and 89% for the control group (n=100).

In case reports, clinical response to ciprofloxacin was observed in 77% (108/141). Evaluation of the rate of bacteriological eradication was feasible in only two of these studies. The first one reported an eradication rate of 100% in 6 cases of neonatal *Enterobacter cloacae* septicemia (clinical response=50%) [21] and the second one a rate of 93% (27/29) in a multi-resistant nosocomial *Pseudomonas* infection (clinical response=83%)[22].

**Epidemiology Data Liverpool Neonatal Unit -** Ciprofloxacin has been used in neonates since the early 1980s motivated by the need to treat suspected (or proven) infection with multi-resistant bacteria in neonates who are very susceptible to infection or to avoid aminoglycosides in patients with compromised renal function while minimizing unit-wide exposure to cephalosporins. The use of ciprofloxacin in Liverpool neonatal units was initially reported by Bannon et al in 1989. [21] Since then over 500 neonates have been exposed to ciprofloxacin. In that time informal clinical surveillance has not yielded any serious adverse events that have been ascribed to ciprofloxacin.

Data from this unit confirms that the minimum inhibitory concentrations for ciprofloxacin lie within the EUCAST breakpoints for sensitivity (≤ 0.5 mg/L) for 91.4% of different isolates of gram negative organisms archived in the microbiology lab between 2004 and 2010 (n=93).

**Neonatal Sepsis Protocol - Liverpool Women's NHS FT (LWFT) Neonatal Unit**

Suspected Infection requires prompt treatment before laboratory confirmation. When clinical features of sepsis in neonates are found a sepsis screen is performed. This includes blood cultures and a lumbar puncture (LP is not routinely done for preterm babies with suspicion of early onset sepsis). Routine blood monitoring includes CRP, differential white cell count and platelets. These aspects of patient management are at the discretion of the clinician and are not included in this protocol.

**Antibiotic Guidelines at LWFT Neonatal Unit**

| Timing | 1st line treatment | 2nd line treatment |
|---|---|---|

| | | | | |
|---|---|---|---|---|
| **Early onset infection** 1st 5 days of life | Benzylpenicillin 25mg/kg 12hourly | Gentamicin mg/kg 36 hourly | 4.5 | Ciprofloxacin 10mg/kg 12 hourly<br>Is added or replaces gentamicin if there is renal impairment or a clinical decision based on lack of clinical response/sensitivities of organism isolated. |
| **Late onset infection** After 1st 5 days of life | Co-amoxyclav 30mg/kg 8 hourly | Gentamicin mg/kg 36 hourly | 4.5 | Ciprofloxacin 10mg/kg 12 hourly<br>replaces gentamicin if there is renal impairment or a clinical decision based on lack of clinical response/sensitivities of organism isolated.<br>Meningitis – **add** cefotaxime 50mg/kg 8 hourly<br>Coagulase Negative Staphylococci (CoNS) – **add** Teicoplanin 16mg/kg load then 8mg/kg 24 hourly |

**NB** these antibiotics are not specified in this study protocol. All medicines given to the participants in this study are entirely at the discretion of the attending physician. In all cases ciprofloxacin is co-prescribed with an antimicrobial targeted at Gram positive bacteria likely to be encountered in neonatal infection.

In practice this means that between 40 and 50 babies a year are treated with ciprofloxacin in LWFT neonatal unit.

Ciprofloxacin is prescribed at Alder Hey Children's NHS FT for hospital acquired infection including pneumonia or central line infections and specific guidelines for cystic fibrosis.

5.2 CIPROFLOXACIN PHARMACOKINETICS AND PHARMACODYNAMICS

**Pharmacokinetics in Adults**

The pharmacokinetics of ciprofloxacin in adults is summarized by Andriole [23] as follows:

Absorption

In adults, oral bioavailability is 60 – 70%. Absorption is impaired by simultaneous administration of cations which bind to the molecule. Oral absorption is not relevant to neonatal sepsis in

Europe, as parenteral administration is required. We are not aware of any studies of absorption of ciprofloxacin in neonates.

### Distribution

In adults the plasma half-life is 3 – 4h, the volume of distribution is 3 – 4 L/kg and plasma protein binding is 20 – 40%. Ciprofloxacin is widely distributed in body water and intracellularly. For example, concentrations within phagocytic cells approach that in plasma. In adults, concentrations in CSF are about half those in plasma, irrespective of the presence or absence of meningitis.

Gous et al. found that ciprofloxacin PK parameters did not differ between adults on intensive care with intra-abdominal sepsis and those with other forms of sepsis. They interpreted this as indicating that fluid shifts did not alter the distribution of ciprofloxacin [24].

### Metabolism

There are four metabolic products of ciprofloxacin. Three of these are microbiologically active (sulfociproprofloxacin, oxociprofloxacin and formylciprofloxacin), while one of them (desethylciprofloxacin) is not active. Formylciprofloxacin, while formed in very low amounts, shows an antibacterial effect equivalent to ciprofloxacin.

Very low concentrations of ciprofloxacin metabolites have been found in human serum and urine. Of an oral dose, 11.3% and 7.5% were excreted as metabolites via the urine and faeces respectively and after an intravenous dose, respectively 9.5% and 2.6%. Metabolism is quantitatively and qualitatively similar after oral and intravenous administrations. There is no information in the literature about the hepatic cytochromes that are implicated in the metabolism of drug.

### Excretion

The liver and kidney are responsible for more than 90% of drug elimination. More than 65 % of ciprofloxacin is excreted unaltered by the kidney. Glomerular filtration and tubular secretion are the main mechanisms of renal excretion. The renal clearance varies between 3 and 5 ml/h/kg and the total clearance between 8 and 10 ml/h/kg

Renal excretion: Renal clearance mechanisms account for approximately two-third of ciprofloxacin elimination. 60% of the unmetabolized ciprofloxacin is eliminated through the kidneys. Renal clearance of ciprofloxacin is 2.7-times higher than the creatinine-clearance, indicating that the drug is excreted by both glomerular filtration and tubular secretion. Non renal clearance: one-third of ciprofloxacin elimination and comprise a combination of hepatic metabolic degradation, biliary excretion, and transluminal secretion across the enteric mucosa.

Ciprofloxacin is actively secreted by kidneys and bile. Studies in rodents have shown that the renal basolateral transporter OAT3 is involved in ciprofloxacin secretion, and that impaired elimination occurs in oat3 null mice [25]. The apical transporter breast cancer related Protein (BCRP) encoded by *ABCG2* contributes to the biliary secretion of ciprofloxacin [26].

## Interactions

The interactions of ciprofloxacin in adults have been summarized by Shakeri-Nejad [27]:

**Binding to divalent and trivalent cations.** This leads to marked reductions in bioavailability of ciprofloxacin if it is co-administered with substances that contain magnesium or iron (but not calcium). This effect is thought to be similar to the binding between magnesium ions and ciprofloxacin that is central to the actions of ciprofloxacin in bacteria. This mechanism of this interaction effect can be extrapolated across age-groups. However, the significance of this interaction in neonates is likely to be low because few neonates are prescribed oral ciprofloxacin. The potential for this interaction will be included in the clinical studies. Compatibility with parenteral nutrition solutions will be assessed during in vitro testing.

**Inhibition of CYP1A2, but not other CYP 450 isoenzymes.** Ciprofloxacin is classed as a moderate inhibitor of CYP1A2 by the FDA [http://www.fda.gov/cder/drug/drugInteractions/tableSubstrates.htm last accessed 19th May 2009]. CYP1A2 metabolizes a limited number of drugs. Of the drugs metabolized by CYP1A2, only caffeine and theophylline are used in neonates. Concomitant therapy with ciprofloxacin and each of these agents is associated with significant increases in the circulating concentrations of both of these agents. Both of these agents have been used in neonates in the past. Recently, theophylline has been superseded by caffeine because caffeine has a much broader safety margin and because of evidence that long-term administration of caffeine can reduce the incidence of bronchopulmonary dysplasia. The expression and function of CYP450 enzymes during development is an area of active research. Caffeine plasma concentration will be assayed during the study when plasma can be scavenged from existing samples for babies receiving caffeine. c) Reduction in levels of phenytoin. This has been reported in one person. If the opportunity arises to examine the effects of ciprofloxacin on phenytoin levels in neonates we will do this.

Considering the role of transporters in the renal excretion of ciprofloxacin, drugs known to interact with SLC or ABC transporters should be studied as potential covariates in the population pharmacokinetic model that will be one of the secondary objectives of this study. With this aim, the drugs received by the babies (and breast feeding mother) will be exhaustively recorded in the patient's chart. At the time of data analysis, each drug will be classified as "substrate", "inhibitor" or "inducer" of the main human drug transporters (according to FDA classification below) and studied as binary covariates (association with an inhibitor : yes/no, with an inducer

y/n,…) http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm#classSub)

Pharmacodynamics

Reviewed in Andriole and Ven Bambeke [28-29]. Ciprofloxacin is a fluoroquinolone with molecular weight 331.4. Fluoroquinolones are bactericidal. They bind to topoisomerases and DNA and disrupt DNA. It is thought that this binding exposes free ends of DNA which trigger apoptosis. The target enzyme varies from species to species with DNA gyrase being the predominant target in Gram negative bacteria and topoisomerase IV being the predominant target in Gram positive bacteria.

Fluoroquinolones display concentration dependent killing and a significant persistent effect / postantibiotic effect [30]. In adults the $AUC_{24h}$/MIC appears to be the best predictor of outcome. When targeted to Gram Negative bacteria animal work suggests that the optimal $AUC_{24h}$/MIC is 125 [31]. When targeted to Gram positive bacteria the optimal $AUC_{24h}$/MIC has been reported to be 40. A high $C_{max}$ and $AUC_{24h}$/MIC > 100 may provide some protection against resistance.

Pharmacokinetics in Children

Minimal data are available in the literature regarding neonates and only a few studies have been conducted that include infants and children (Appendix 1). Drug pharmacokinetics in neonates differs from other age groups[5]. Preterm babies who are particularly vulnerable to neonatal infections, can present notable differences because of higher extracellular fluid volume, immature renal and hepatic functions at birth and post-natal maturation of these organs. **(Appendix 1 PK data in infants and children).**

Lipman et al. [6] examined ciprofloxacin PK in 20 children (3 months -5 years) on PICU given an intravenous dose of 10mg/kg 12 hourly as a one hour infusion. In 10 infants aged between 3 months and 1 year the peak concentrations ($C_{max}$) on $D_0$ were 6.1±1.2 mg/L, $D_2$ 9.0±1.8 mg/L and $D_7$ 5.8±1.3 mg/L. The $AUC_{12h}$ was estimated to be around 15-20 mg/L.h. This suggests that the $AUC_{24h}$/MIC would only be above 100 for bacteria with an MIC < 0.3mg/L. As MIC for gram negative pathogen may be higher (up to 0.8 mg/L for some strains of Ps. Aeruginosa [5, 31-32], this dose may be too low. However, the authors did not studied infants < 3 months in whom elimination may be impaired, leading to higher exposure. Results were similar in children aged 1 year to 5 years.

Bannon et al. treated 6 preterm neonates (24-29 weeks) with E. cloacae septicemia using intravenous ciprofloxacin at a dose of 10 mg/kg/day (divided into 2 administration, i.e. 5 mg/kg 12 hourly) and measured peak (30 min after injection) and trough concentration following the third administration [21]. The mean peak concentration was 3.1 µg/mL (range 1.45 to 5.7 µg/mL)

after the third dose and mean trough level was 1.25 µg/mL (range 0.04 to 2.6 µg/mL). Aggarwal et al. [32] used intravenous ciprofloxacin at a dose of 10mg/kg/dose twice daily (20 mg/kg/day) in 24 Indian preterm neonates (28-36 weeks) having severe infections of variable aetiologies. [32]. In this study, peak and trough ciprofloxacin levels at 1, 3 and 7 days of treatment were comparable and no differences were observed between the <1500g and >1500g of weight and the <7 days and >7 days of postnatal age sub-groups, with respect to corresponding peak and trough levels on sampling days. Of note, estimated clinical cure rate was 67% and no adverse events were observed during the use of ciprofloxacin. At day 3 (as comparison with Bannon's study [21]), peaks ranged 0.1 – 7.3 mg/L and trough ranged 0.0 – 2.1 mg/L. Steady state levels were reached from the 1st day, and there was no evidence of accumulation over a week's duration.

Goepp et al. [33] studied a single infant with ventriculitis, who received 35 mg/kg/day of ciprofloxacin. The peak serum level was 11.6 µg/mL after the 6th dose, but the trough level (0.9 µg/mL) was similar to that in Aggarwal's study [32].

Wessalowski, et al.[34] reported serum levels of 0.1 to 2.8 µg/mL in random serum samples drawn from one term neonate, who had a brain abscess (dose not reported).

Van den Oever et al. [35] reviewed the pharmacokinetics of ciprofloxacin in 7 preterm infants, less than 30 weeks gestation, reported from various studies. Intravenous doses ranging from 4 to 40 mg/kg/day yielded adequate serum peak concentrations (0.98 to 5.7 µg/mL), but trough-to-peak ratio were high (median 32%), suggesting slow elimination in preterm babies.

These studies concluded that ciprofloxacin was an effective treatment of neonatal sepsis. They indicate that higher doses might be required for treating infections with less sensitive strains like Pseudomonas aeruginosa (MIC 0.8 mg/L). **However, none of these studies provide data on individual AUC that could be extrapolated in terms of AUC/MIC.** Peak concentrations were similar to those found in adults. However, elimination is dependent on renal function and elimination is expected to be slower in neonates than older children and adults. In adults, intra-abdominal sepsis was not associated with altered PK parameters implying that fluid shifts did not alter the handling of ciprofloxacin [24], but this issue has not been examined in neonates.

Some PK studies have been conducted in cystic fibrosis (CF) patients but will not be reported here since important PK differences exist as compared to non CF children.

**Pharmacokinetic Models -**In pharmacokinetic studies, a sufficient number of samples, taken at adequate times are of great importance to provide a reliable estimation of PK parameters. Samples obtained in routine clinical practice may be inadequate (e.g. sampling during the infusion of the drug), or, more often, not well-balanced across the dosing interval. The non-optimal sparse sampling schedule can bias the estimates of clearance and volume of

distribution. For example, sampling too late after end of infusion can underestimate clearance if the first compartment of distribution is missed. A modelling study conducted with theophylline concluded that observational study designs with only 20 premature neonates and unbalanced sampling was inadequate to allow for precise estimation of theophylline population PK parameters. (ref Y. Zhang et al. : Simulation-Based Sample Size Optimization for Population Pharmacokinetic Studies in Premature Neonates www.aapsj.org/abstracts/AM_2009/AAPS2009-001792.PDF).

The ideal option would have been to select an “optimized” sampling schedule, based on D-optimal strategy. However, this is not applicable because we do not have the preliminary PK information in neonate and young infants (typical PK parameters, variability, covariate etc) to optimize such a sparse sampling schedule.

Another option is to select a priori a sampling schedule which will ensure reliable estimation of PK parameters and limit the number of samples to a maximum of 3 on the first and last day of treatment (section 6.13).

Pharmacogenetics

Pharmacogenetic variability together with variable factors` contribute to the variability in individual risk/benefit ratios. In neonates, the pharmacogenetic underpinnings together with the developing activity of drug metabolizing enzymes and transporters contribute to the individual and age dependent capacity to metabolize and eliminate drugs. Ciprofloxacin is eliminated more than 65% unchanged by the kidney. Metabolism takes place to 3 active and one inactive metabolite but the liver enzymes involved have not yet been identified. Since ciprofloxacin is not extensively metabolized, genetic polymorphism in metabolizing enzymes of ciprofloxacin to single metabolites are not likely to influence the overall efficacy or toxicity of ciprofloxacin.

Elimination and target distribution of ciprofloxacion might be influenced by individual activity of active drug transporters. Ciprofloxacin is transported by organic anion transporter 3 (OAT3) and by the BCRP transporter, both transporters being genetically polymorphic. Thus, individual differences in efficacy and in elimination might partly depend on genetic polymorphisms of active drug transporters [25-26, 36].

In addition to pharmacokinetics, pharmacogenetic polymorphisms might affect safety and efficacy of drugs. Ciprofloxacin is active in the central nervous system, and sometimes causing psychotic symptoms as side effects, known to be partly mediated by inhibition of the GABA A receptor [37]. Genetic variants in the GABA A receptor therefore might modulate the susceptibility to central nervous side effects of ciprofloxacin.

Some pharmacologically relevant gene polymorphisms divide the population into large group such as halves or thirds. Pharmacogenetic data will be used to explore whether any such prominent differences in genotype are associated with differences in variation in ciprofloxacin PK. More definitive evaluation of genotype-phenotype correlations will come from the registry study which will have a genetic component.

In addition to specific drug targets, genetic variability is known to contribute to the individual susceptibility to septicaemia. Polymorphisms in genes like *Toll-like receptor 4, Il-6*, *Il-10, CD14*, as well as hemostasis genes might contribute to the severity and course of sepsis and therefore influence the individual benefit of the treatment with ciprofloxacin.

*Justification for the study*

In order to support prescribing decisions relating to ciprofloxacin in neonates, it is necessary to gather more information about:

PK variables

Dosage regimen

Short Term Safety

Licensing support

**PK Variables:** There have been no reports of PK in infants less than 3 months old that include an assessment of the key PK/PD parameter, the $AUC_{24h}$/MIC. CSF penetration of ciprofloxacin has not been reported in neonates or young infants.

**Dose Regimen** - the daily dose reported in the studies involving neonates included in the systematic review by Kaguelidou et al. varied from 5mg/kg to 60mg/kg. The results from Lipman et al. [6] suggest that a dose greater than 10mg/kg/12h would be required for optimal pharmacokinetics in infants 3 months-1 year of age. However, this dose may be sufficient in neonates who have impaired renal function. However, given the PD characteristics of ciprofloxacin a detailed description of exposure including $AUC_{24h}$ is required to optimize the dosage regimen in neonates and young infants. None of the published studies in neonates < 3 months have provided such data

**Short Term Safety**: A number of adverse events have been associated with ciprofloxacin. These are summarized in the Summary of Product Characteristics for marketed products. A generic ciprofloxacin SPC is included as Appendix II. This study will document adverse Events occurring during treatment and during a 3 day washout period will be recorded in this study.

Safety issues, including those occurring g after the 3 day washout period, will also be studied in the TINN Registry Study. Participants in this study will be eligible for the registry study.

The most prominent concern in children has been the possibility of arthropathy. The WHO Subcommittee on Essential Medicines for Children recommended that more data about safety in neonates is needed but noted *"a summary of findings in children (31 reports, > 7000 children) where arthropathy was found to be reversible, without long-term sequelae, and not convincingly correlated with the use of fluoroquinolones in children."* [3].

**Licensing Support** To provide data required by the European Medicines Agency for a Paediatric Use Marketing Authorisation consistent with the EU Paediatric Regulation 2006 [1] to ensure that a medicinal product for use in children undergoes extensive studies to ensure that it is safe of high quality and for use in the target population.

Study Design

*Objectives*

**Primary Objective**

To evaluate the multiple-dose pharmacokinetics of ciprofloxacin in neonates and infants 24 up to 52 weeks postmenstrual age with suspected (or proven) Gram negative infection

**Secondary Objectives:**

To evaluate the tolerability[1] of ciprofloxacin in neonates with suspected (*or proven*) Gram Negative infection.

To describe short-term safety

To describe the outcome of treatment episodes

---

[1] We use the standard definition of tolerability: "The tolerability of the medical product represents the degree to which overt adverse effects can be tolerated by the subject". This is taken from the Glossary of ICH E9 "**Statistical Principles for Clinical Trials"**

http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002928.pdf (last accessed 28th August 2010).

*Endpoints*

**Primary Endpoints:**

Ciprofloxacin plasma concentration and population pharmacokinetic (PK) parameters [maximum concentration, clearance, area under the curve (0-tau)], their relationship with selected covariates their interindividual variability (CV%).

Covariate analysis will include postmenstrual age, gestational age, postnatal age, weight, and serum creatinine on the day of the first PK sample of each period (D1 and D5-7).

**Secondary Endpoints:**

**P**K variables, including apparent volume of distribution and half life.

Withdrawal due to lack of tolerability as judged by attending clinicians.

Adverse events (AEs) and serious adverse events (SAEs).

Reasons for stopping ciprofloxacin and changing or changing to other antibiotics with nature of other medicines.

Outcome of treatment episodes (clinical and microbiological) –Test of Cure

Data will also be collected for **covariates** that may cause between-subject variability:

Demographic (postmenstrual age, gestational age, post conception age, birth weight, postnatal age, current body weight, gender).

Clinical (serum creatinine, albumin, total bilirubin, conjugated bibirubin, hemoglobin, hematocrit, AST, ALT and marker of severity of illness)

Co-medication - (including cations and caffeine)

Treatment – surgery, ventilation and transfusion

Necrotising Enterocolitis

Pharmacogenetics - major transporters implicated in ciprofloxacin disposition and clearance

*Time Frame* - ***recruitment will commence February 2011 until 2013.***

*Assessment of outcomes*

The MIC data for ciprofloxacin in Gram Negative bacteria isolated from participants in our studies will be obtained (this will be a small percentage of those recruited). This will allow us to estimate $AUC_{24h}$/MIC for common Gram negative infections and indicate whether achieved circulating concentrations are likely to be sufficient.

This study is not large enough to enable a definitive assessment of the safety of ciprofloxacin. The physiological changes in development stratified from 24 - 52 weeks gestation would require large numbers of babies receiving ciprofloxacin. The study will establish initial, short term safety data. The TINN ciprofloxacin registry that includes other European Centres will describe safety data.

*Study Population*

**Recruiting Sites:** Participants will be recruited on a neonatal unit, paediatric wards or the paediatric intensive care unit at Liverpool Women's NHS FT and Alder Hey Children's NHS FT that share services for neonates including laboratory services for neonates. Recruited neonates who are subsequently transferred to other hospitals having completed ciprofloxacin treatment will be followed up by collaborators at approved step down sites across the region.

**Sample Size:** Neonates and infants (n=50) will be recruited and stratified between 24 up to 52 weeks post menstrual age. The target recruitment is 5 - 8 patients per 4/5 week period depending on the number of patients available. The minimum acceptable recruitment is 10 patients within two adjacent time periods (8 weeks), recruitment will ensure there are at least 10 patients under 32 weeks; 20 below or above 36 weeks; and 10 above 44 weeks.

<table>
<tr><td colspan="7">Recruitment target (n=50) aims to represent each postmenstrual age (PMA) group between 24 -52 weeks stratified over 4/5 week periods:</td></tr>
<tr><td colspan="4">Pre -term</td><td>Term newborn infants</td><td colspan="2">Infants > 28 days to 3 months</td></tr>
<tr><td>24-27</td><td>28-31</td><td>32-35</td><td>36-39</td><td>40-43</td><td>44-47</td><td>48-52</td></tr>
<tr><td colspan="7">The target recruitment is 5 - 8 patients per 4 week period but a minimum of 10 patients will be accepted within two adjacent time periods (8 weeks).</td></tr>
</table>

This is consistent with the EMEA age classification for the clinical investigation of medicinal products in the paediatric population (CPMP/ICH/2711/99) preterm newborn infants, term newborn infants (0-27 days) infants and toddlers 28 days -23 months [5]. As this study is

designed for neonates and young infants the age is limited to a maximum of 3 months corrected gestational age.

This is not a hypothesis-testing study. The pharmacokinetic (PK) data generated from this study will assist in dose selection for subsequent studies and for labelling purposes. So, no formal power calculation has been used to determine the sample size.

*Inclusion Criteria*

Prescribed ciprofloxacin for clinical care based on the unit's clinical protocol for managing sepsis. As decided by the physician caring for the baby. All other medication will be at the discretion of the attending physician.

Post menstrual age 24 – 52 weeks

Parental consent to participate.

*Exclusion criteria*

Likely not to survive for more than 48 hours in judgement of attending physician.

Ciprofloxacin commenced before 5$^{th}$ day of life (excluded due to immature renal function)

Babies may also be recruited to other studies unless this would require additional blood samples during the same sampling period above the maximum allowed by the EMEA / MCRN guidance for blood sampling for neonates /children in research.

*Subject withdrawal*

Ciprofloxacin stopped due to clinical decision before day 5 of a course of ciprofloxacin

Transfer to another unit before day 5 of ciprofloxacin (excluding transfer between recruiting sites)

Parental request

In these cases data obtained up to the point of withdrawal will be used in the analyses.

*Consent* [1]

Consent to this study is to allow detailed monitoring of the babies (receiving ciprofloxacin as part of their clinical care) and access to medical notes.

**Consent for the Pharmacokinetic Study** is to allow the research team to commence:

Detailed monitoring of the baby

Collection of samples at specific times (blood and faeces)

Access to the baby's medical notes or clinical data

**Further, specific consent** is also requested for additional samples:

Cerebrospinal Fluid Sample – an additional sample when required clinically

DNA sample for pharmacogenetic analysis

The parent information sheet and researcher will explain that the risks are minimal and specific to sample collection and use of data.

The decision to start ciprofloxacin will be made by the attending physician in accordance with standard unit practice as part of clinical care. Consent is specifically for monitoring babies not for the treatment with ciprofloxacin. Any side effects of the drug relating to their clinical care have therefore not been included in the consent process. As with many drugs in paediatrics ciprofloxacin is used off-label in this age-group. The participating institution has considerable experience of this medication and does not consider it a novel or experimental treatment. It is not routine practice to discuss off-label medication use with patients.

**Stages of Consent Process** - to ensure parents have ample time to reflect on the information relating to a study before making an informed decision about their baby participating in the clinical trial a continuous consent process will include 3 approaches:

Flyer/Leaflet

Formal Informed Consent Process

Continuous Consent Process

To ensure parents are given ample time and are approached at a time that is less stressful for them we aim to approach them prior to the baby becoming eligible and when the neonate / infant is judged to be stable enough by the clinical care team. An important issue is the need to seek informed consent within the time available to allow a PK sample to be taken at the end of the first infusion of ciprofloxacin. Parents are not present on the unit in a predictable manner. Many neonates are transferred significant distances, parents have to care for other children, find visiting distressing and mothers are unwell in the days after birth. By requesting consent prior to the baby becoming eligible (prior to ciprofloxacin being prescribed) we will allow families ample time to make an informed decision whilst avoiding any delay in commencing the monitoring.

**Flyer/Leaflet** (Appendix III) **-**The initial approach to parents of **any** baby under 3 months of age will be to routinely advise them about the potential for recruitment to the study by providing a flyer as a brief introduction to the study. This will also be displayed in public areas.

**Formal Consent** - detailed information as part of the formal consent process will then be provided to families of babies who are more likely to be prescribed ciprofloxacin as part of their care. The following groups will be approached for formal consent:

**a) Neonates / infants** screened for sepsis due to presenting clinical signs (these babies will be started on different antibiotics but some of them will be changed to ciprofloxacin because of their clinical course (section 5.1).

**b) Neonates with suspected (or proven) Gram negative bacteria**

Prescribed the first line antibiotics for Gram negative infections

**Neonates / infants at high risk of infection –** those born before 28 weeks post menstrual age.

**Informed consent:** a parent information sheet (Appendix IV) will be provided and used to structure a discussion with the parent(s). Parent(s) will be allowed ample time to make their decision. They will be asked how long they would like to consider the information and what time is convenient for them when the researcher may return to answer any questions they may have. At the second discussion they will then be asked if they need more time to make a decision or if they feel that they are sufficiently informed to make the decision. Consent will be sought before any procedures that are not part of routine clinical care are carried out.

Administration of ciprofloxacin will not be delayed by the consent process. If parents or designated legal representative have not been able to make a decision by the time the first blood sample is required the child will not be enrolled in the study.

**Continuous Consent** - The study team will adopt a process of “ongoing consent” during which the progress of participants and the study will be discussed, parents will be given repeated opportunities to ask questions, clarify any issues about the study and confirm or withdraw their agreement to their child participating the study [38]. The aim of this process is to optimize the comfort of parents with the study when the time comes for blood sampling, OR, to give parents the opportunity to withdraw consent if they feel that that is appropriate.

**Consent Forms** – 3 copies of the signed consent form (attached to the information sheet) 1) parent 2) Case Report File 3) Medical notes (Appendix V).

**Parental Responsibility** - Consent will be obtained from a person with parental responsibility defined by the Children's Act 1989 and the Children and Adoption Act 2002.

**Research Team –** the informed consent process will be delivered by health care professionals with suitable paediatric /neonatal experience and training, including GCP[39], and who are delegated this role by the Investigator and listed on the delegation log.

**Other studies –** neonates who have been recruited to other trials will be eligible for this trial unless the Investigator deems that one trial would interfere with the validity of the other trial. Recruitment to this trial will not preclude recruitment to other trials. This is subject to parental consent.

Recruitment:

When a clinical decision to start ciprofloxacin has been made AND consent is in place AND medical eligibility has been confirmed, a neonate / infant will be recruited to the study and given a study number. This is consistent with ICH GCP [39].

**Determining Eligibility** - following consent the medical suitability for recruitment will be confirmed by a physician listed on the delegation log and written in the notes to state the patient meets the eligibility criteria prior to any study procedure (required by the regulatory body MHRA).

**Promotion/Transparency** – Information sheets, brochure, posters and other material will be approved by the Ethics Committee.

**Screening**: A log will be kept of all eligible babies detailing which parents/babies are approached and which babies are recruited. When babies are not recruited a reason for this will be recorded, including the comments of parents if they wish to make a comment.

*Benefits and Risks of the PK Study*

The risk /benefit evaluation of study-specific procedures depends on whether the participant could derive any benefit from the study and the extent of risk arising from each element of the study. In this study, the participants will not gain any benefit since the assays will be sent to a different centre for batch analysis The results of the assays will not be available to the clinicians during the course of ciprofloxacin and so there will be no opportunity for "therapeutic drug monitoring" to come from the study. Thus, the only benefits are to other babies.

The guidance from the UK Royal college of Paediatrics states 'where children are unable to give consent to taking of blood for non-therapeutic purposes by reason on insufficient maturity or understanding their parents or guardians may consent to the taking of blood for non-

therapeutic purposes provided they have been given and understand a full explanation of the reasons for blood sampling and have balanced its risk to their child' [40] Sampling blood for non-therapeutic purposes, provided appropriate consent is given on behalf of the participant, is also recognized in the USA, see US 21 CFR 50.51 and 50.53 as discussed in [41].

"Blood taken by a skilled person poses only a minimal risk of physical harm, except *possibly* the taking of a separate sample of blood from a very immature infant' (a commentary on the RCP guidance) [40] This refers to both the volume of blood and the sampling procedure:

**Blood Sampling Procedure:** The aspects of the procedure to be considered are: pain/distress, and impact on future procedures due to loss of potential sites for vascular access. To minimize the risk of distress and harm we will liaise closely with the clinical team and parents. Septic babies require frequent bloods to monitor blood gases or blood glucose therefore the timing of sampling will be co-ordinated closely with the clinical requirements and babies cares.

Blood will be taken by those skilled in sampling from neonates. When possible, an arterial or intravenous line that is suitable for sampling blood will be used. When there is no suitable indwelling line the clinician will make a choice between venepuncture and capillary sampling based on the available opportunities for sampling. There are a range of options to minimize pain and distress, see "Points to consider when planning the collection of blood samples in clinical trials of investigational medicinal products." http://ctuprod.liv.ac.uk/mcrnweb/images/mcrn_guide_for_blood_sampling_v1.0.pdf (last accessed 25th January 2010) from the UK National Institute of Health Research Medicines for Children Research Network. Topical anaesthetics are not relevant for preterm infants.

**Volume of Blood:** As noted in Section 6.13, we will work within limits for blood volume for neonates proposed by the European Medicines Agency [5] and Medicines for Children Research Network Guidance [42]. Additional routine clinical samples will be scavenged at various time points to minimize study specific procedures. These may occur at suboptimal time points for PK analysis. Therefore specific sampling time points are also be required.

Observational data exists to support the idea that the parents and professional carers of at least some neonates rate the pain and distress of study-specific blood sampling episodes to be minimal [43]. Fifty four of 90 parents (60%) reported that their term neonates was "not at all upset" by a non-clinically indicated study-specific blood sampling episode [43]. PK studies that require additional samples from neonates, to the clinically indicated sampling episodes have previously received favorable opinions from UK Research Ethics Committees.

**Cerebrospinal Fluid PK Sample** – will only be collected if the baby requires the sample for clinical purposes and when a further 0.5 ml can be collected from the same sampling episode.

**Access to Personal Identifiable Data** (section 7.6) relating to the study will all be collected in the course of routine clinical practice and so there are no risk issues beyond loss of confidentiality. Confidentiality will be maintained in accord with standard practice in the health care setting. Data that leaves the health care setting will be protected by "linked anonymisation" so that the data will not be associated with any personal identifiers once it leaves the health care setting. In this way, the risks relating to data will be no greater than routine clinical practice. Data up to the time of withdrawal will be included unless parents request that data is discarded (consent form will state this).

*Baseline Assessments following recruitment:*

a clinical assessment will consist of a standard physical examination conducted by a health care professional with appropriate clinical training and experience and study-specific training who appears on the delegation log. Blood Results from haematology, biochemistry and blood gas analysis that are required for clinical care.

Biochemistry[2]: Plasma creatinine, $Na^+$, $K^+$, $Cl^-$, AST, ALT, alkaline phosphatase, total bilirubin, conjugated bilirubin, albumin, total calcium, corrected calcium, magnesium

C-reactive protein

Full Blood Count[3]: Hb, total white cell count, differential white cell count, MCV. MCH, MCHC and platelets.

Blood gas[4]: glucose, lactate, ionized calcium[2]

Microbiological [5]- clinically indicated samples including blood cultures and lumbar punctures carried out for patient's care before the start of ciprofloxacin therapy will be reported.

A faeces sample will be taken after recruitment when available.

*Dosage and Administration*

Dosing schedule: 10mg/kg 12 hourly infused intravenously over 30 - 60 minutes

[2] To be measured using near-patient testing on participating units

The dose selected for our study is based on previous studies in neonates and infants and is consistent with UK BNF for Children[44]. Although only few PK data are available in neonates for this drug, we can assume that neonates have reduced clearance and increased volume of distribution compared with the adult population due to their profound changes in term of physiology and organ maturation. (cf 5.4). Previous studies have demonstrated that plasma $AUC_{0\text{-}24h}$/MIC ratio (AUIC) was a good predictor of efficacy in adults in the treatment of serious gram-negative infections. As we are not aware of any evidence that ciprofloxacin pharmacodynamic in neonates will differ from those in adults, the AUC/MIC target of 100 was chosen as efficacy predictor (cf 5.2.2).The only data available in preterm neonates are from Aggarwal et al. [23] but this study did not evaluate AUCs. Lipman et al. [21] determined the pharmacokinetic profile of ciprofloxacin (10mg/kg administered 12 hourly) in 10 patients from 3 months to 1 years. By dividing the AUC0-24h by a MIC of 0.25 mg/l, the AUC/MIC is close to 120 in this group of patients. In cases where MIC would be higher (e.g. 0.8 mg/L), the AUC/MIC would be only 37, which is much lower than the target value for Gram negative pathogens. However, systemic exposure may be higher in neonates < 3 months.

## Dose Adjustment

Data from a recent study of the Minimum Inhibitory Concentration of Ciprofloxacin for Gram negative isolates in the participating Neonatal Unit suggests the current dosage is sufficient to achieve the EUCAST sensitivity breakpoint in the majority of cases.

Interim analysis of PK samples will be performed.

An increased dose will be envisaged if AUC/MIC is below the expected target of 100. As the PK is linear, we would propose a “rule of proportionality”.

If an adjustment is required the nature of the adjustment will depend on the spread of results. In the absence of extreme variation we will follow the following rule:

“If more than 25% of patients exhibit an AUC/MIC < 80, the dose will be increased by 20%. If AUC/MIC<50 the dose will be doubled.”

The choice of dose adjustment would be guided by review of the data by the TINN consortium.

Dose **decrease** would only be supported if acute side effects occur but this is not expected. In such a case, the safety monitoring board would decide what to do.

## Drug Administration

A commercial, generic preparation will be used containing 2 mg/ml of ciprofloxacin. Ciprofloxacin provided from the hospital pharmacy supplies will be administered at a dose of 10

mg/kg 12 hourly. Infusion will be prepared and administered according to routine clinical practice by central or peripheral intravenous infusion according to the vascular access available for that child over 30 - 60 minutes.

*Pharmacokinetic Samples and Assessment*

(see Standard Operating Procedure for storage, labelling and couriering of samples)

**The maximum number of study specific blood samples is limited to 3 per baby and per period (Day 1 and on one day between Day 5- 7) (2 samples per day for babies less than 1000g). In order to completely cover the distribution and elimination phase in the whole population, babies will be assigned to 1 of the 2 predefined 3-time (or 2-time) point schedules.**

Sample time schedule - **babies will be assigned to one of the two groups of sampling times (A and B) as follows:**

| **Group** | **Weight** | **Sampling times on day 1 and day 5 -7 or last day of ciprofloxacin treatment*:** | | | | | **Maximum blood volume for all study samples** |
|---|---|---|---|---|---|---|---|
| **A** | > 1000g | | T1 | T3 | T8 | | 1.2 ml |
| | | Ai | T1 | T3 | | | |
| | < 1000g** | | | | | | 0.8 ml |
| | | Aii | T1 | | T8 | | |
| | | Aiii | | T3 | T8 | | |
| **B** | > 1000g | | T2 | T6 | | T12 | 1.2 ml |
| | | Bi | T2 | T6 | | | |
| | < 1000g** | Bii | T2 | | | T12 | 0.8 ml |
| | | Biii | | T6 | | T12 | |

* The sampling times are **calculated from the start** of the first infusion of ciprofloxacin **except** for T1 when the sample must be taken immediately after the end of the first infusion i.e at the end of the 30 minute infusion or the end of the 60 minute infusion.

Samples are required on day 1 of starting ciprofloxacin and completed on one day between days 5-7 of ciprofloxacin treatment to allow some flexibility but day 7 is preferred when possible.

** Babies less than 1000g will have samples taken on alternate times to minimise blood loss but allocated to one of three subgroups and 2 specific times to ensure representation of each period.

Sample Time Window: **The T1 sample will be taken within 3 minutes of the end of the infusion. The infusion is either given over 30 minutes or 60 minutes according the local practice. All other sample times (T) periods represent are the number of hours after the infusion started.**

To ensure the samples provide accurate data all other samples are required within a 20 minute interval of the allocated sampling time (10 minutes before or after the allocated time).

The research team will discuss the timing of samples required with the clinical team and parents to ensure cares are co-ordinated at this time and with minimal disruption to the baby's routine. However, some additional blood sampling episodes will be required and this will be made clear during the consent process.

Scavenged samples: **Additional, opportunistic samples will add to the precision of the pilot PK model. Blood that has been taken for clinical monitoring will be scavenged throughout ciprofloxacin treatment and up to 24-48 hours following treatment to monitor liver and renal clearance.**

Blood Sample volume: **The assay requires 200 microlitres of blood collected in an EDTA bottle. The sampling schema requires a total of 0.4 - 0.6 mls of blood on the first and last day of treatment a total per patient maximum of 0.8 - 1.2 mls of blood within 7 days depending on the babies weight. For infants < 1000g we will use alternate sampling time points and each infant < 1000g will contribute randomly two among the three time points a maximum of 0.8 ml of blood within 7 days. For a 500g infant with estimated circulating volume of 90 ml/kg or a circulating volume of 45mls, 3% would be 1.35 ml). This is consistent with the EMEA and Medicines for Children Research Network sampling of neonates in clinical trials, that recommends 1% of blood loss on each occasion and 3% over 28 days.**

Blood Sample Collection and Source: **All blood sampling episodes will be undertaken by clinicians with neonatal skills and experience. Efforts to minimise pain and distress will be tailored to the individual child by clinical staff with the involvement of parents. Sample source - Blood will be sampled from one of the following sources in the light of clinical circumstances as judged by the health**

**care team and investigators. Samples will be taken from a different vascular access route to the one used for administration. This list provides the order of preference for site of blood sampling.**

Umbilical arterial line

Peripheral arterial line

Umbilical venous line

Surgical central line (Broviac)

Capillary sampling

Venepuncture

Venous cannula

When venepuncture or capillary sampling is required each sampling episode will involve a maximum of 2 (two) attempts to obtain the sample, when each attempt is defined as a break of the skin of the participant.

When a sample is taken via an indwelling line (surgical, umbilical or peripheral) sufficient serosanguinous fluid will be taken to account for dead space.

Cerebrospinal fluid (CSF) Pharmacokinetic Sample:

If the baby requires a clinically indicated CSF sample and consent is obtained for the CSF sub-study, a further 0.5ml will be taken after the CSF required for clinical tests has been collected.

CSF may be obtained by the following methods

a. Lumbar puncture b. Ventricular tap c. CSF reservoir tap

The sample will be collected in a sterile polypropylene tube in one of 2 ways; either at the time that CSF is collected for diagnostic purposes or a scavenged sample from the clinical microbiology laboratory

**Pharmacokinetic Analysis**

(see Standard Operating Procedure)

An analytical Liquid Chromatography-mass Spectrometry (LC-MS) method for ciprofloxacin detection in human plasma suitable for assaying the small plasma volumes obtainable in neonates has been developed and implemented in the pharmacology laboratory (University Hospital of Tours, France).

*Pharmacogenetic samples:*

For pharmacogenetic analyses, DNA will be extracted from the cells left over after centrifugation of the plasma EDTA samples to reduce the amount of blood taken from the neonate therefore no additional sample will be required. The Quieten method for DNA extraction will be used. DNA will be extracted at University of Liverpool laboratory based at Liverpool Women's Hospital.

**Following a blood transfusion** - No further blood samples may be used for DNA. In this situation buccal samples will be collected using a swab in the baby's mouth.

**Pharmacogenetic analysis** will be undertaken at the Institute of Pharmacology of Natural Products and Clinical Pharmacology University of Ulm Germany where they will be stored for a maximum of 10 years.

*Microbiological Samples*

The results of all blood cultures and CSF cultures will be recorded. Microbiological Analysis will be carried out in the Clinical Microbiology laboratory at Royal Liverpool University according to their Standard Operating Procedures. The MIC values will be obtained for all positive gram negative cultures. Each faeces sample will be inoculated onto 4 plates (1 plain and 3 incorporating CIP at 0.1, 1.0 & 4.0mg/l). Only organisms that grow above 0.1mg will be characterized.

**Faeces Samples -** A basal sample of faeces will be collected to assess colonization at anytime following consent (requested by PDCO). A further sample will be collected at the end of treatment with ciprofloxacin and between weeks 4 – 6 following completion of the antibiotic to test for gram negative insensitivity. If the patient has been discharged then the families will be asked to bring a sample to clinic, or the family may post the sample to the laboratory in an approved container (consistent with Health and Safety Executive Regulations).

*Test-of-cure*

Clinical Cure will be defined as CRP < 10mg/L three days after the cessation of ciprofloxacin. Data will be collected on the duration of ciprofloxacin therapy including whether ciprofloxacin was stopped because of incompatibility. Three days after the cessation of ciprofloxacin treatment a detailed assessment of clinical outcome will be conducted, comprising:

Clinical: a physical examination conducted by a health care professional with appropriate clinical training and experience and study-specific training who appears on the delegation log.

Laboratory: C Reactive Protein

Microbiological: results of any repeat blood cultures.

*STUDY SAFETY MONITORING*

During ciprofloxacin therapy the study team will conduct a systematic daily review of patient records until 3 days following the last dose using electronic patient data system and consult carers and parents in order to identify adverse events. Pharmacovigilance reporting will commence will continue until 6 weeks following the treatment.

**Clinical Blood Samples** - All Biochemistry / Haematology analyses carried out during the course of ciprofloxacin for routine care will be assessed for safety parameters. No study-specific blood sampling episodes will be undertaken for safety purposes.

Laboratory Reference Ranges **(all clinical samples are processed at Alder Hey Children's NHS FT for both recruiting sites).**

**Haematology - Full Blood Count**

| **Value:** | **Range for age group:** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Day 1** | **Day 3** | **Day 7** | **Day 14** | **1 month** | **2 month** | **6 month** |
| Hb | 14 - 22 | 14 -22 | 13 -21 | 12.5 - 20.5 | 10 -17 | 9 -13.5 | 10 -14.1 |
| MCV | 96 -130 | 94 -130 | 88 -120 | 86 - 120 | 85 -120 | 77 -115 | 72 -95 |
| MCH | 31 -39 | 30 -37 | 28 -37 | 28 -37 | 27 -36 | 29 -34 | 25 -33 |
| MCHC | 30 -36 | 30 -36 | 28 -36 | 28 -36 | 29 -36.5 | 29 - 36.5 | 30 -36 |
| Platelets | 150 -400 | 150-400 | 150-400 | 150-400 | 150-400 | 150-400 | 150-400 |
| Total WCC | 9 -1.84 | 9 - 18.4 | 5 -18.4 | 5 -18 | 5 -18 | 5 -18 | 5 -17 |
| Haematocrit | 45 -67 | 45 -67 | 42 -66 | 39 -63 | 31 -55 | 28 -42 | 30 -41 |
| Differential WCC:<br>Neutrophil<br>Lymphocyte<br>Monocyte<br>Eosinophil<br>Basophil<br>Myelocytes | 4.8 - 17.1<br>2 - 7.3<br>0.06 - 1.9<br>0 - 0.8<br>0 - 0.2<br>0 - 0.1 | 2 -94<br>2 -7.3<br>0.06 -1.9<br>0 -0.8<br>0 -0.2<br>0 -0.1 | 1.8 -8<br>2.8 -9.1<br>0.06 -1.7<br>0 -0.9<br>0 -0.2<br>0 -0.1 | 1.7 - 6.0<br>2.8 - 9.1<br>0.06 - 1.7<br>0 -0.8<br>0 -0.2 | 1 -9.0<br>3 -13.5<br>0.06 -1.7<br>0 -0.6<br>0 -0.2 | 1 -9<br>3 - 13.5<br>0.06 -1.7<br>0 -0.8<br>0 -0.2. | 1 -8.5<br>4 -13.5<br>0.06 -1.3<br>0 -0.8<br>0 -0.2 |

**Biochemistry**

| **Blood Sample:** | **Age:** | **Range:** |
|---|---|---|
| Creatinine | 2 weeks<br>2-26 weeks | 30 -58 µmol/L<br>20 -48 µmol/L |
| Sodium | 0-1month<br>> 1month | 132 – 142 mmol/L<br>135 – 145 mmol/L |
| Potassium | 0-1month<br>> 1 month | 4.0 – 6.2 mmol/L<br>3.5 – 5.5 mmol/L |
| Chloride | | 95 – 105 mmol/L |
| AST | 0-1month<br>> 1 month | 23 – 73 IU/L<br>15 – 58 IU/L |
| ALT | 0-1month | 9 - 44 IU/L |

| | | |
|---|---|---|
| | > 1 month | 9 - 36 IU/L |
| ALK Phosphate | 0-1month<br>>1month | <1600 IU/L<br>187 - 1197 IU/L |
| Total bilirubin | 0-1 month<br>> 1month | <340 µmol/L<br><15 µmol/L |
| Conjugated bilirubin | 0-1month<br>>1 month | <10 µmol/L<br><10 µmol/L |
| Albumin | 0-2 months<br>> 2 months | 30 - 45 g/L<br>37 - 53 g/L |
| Total Calcium | 0-1week<br>1 week - 2 years | 1.75 – 2.99 mmol/L<br>2.20 – 2.79 mmol/L |
| Corrected calcium | | For albumin <40 g/L : = measured Ca + 0.02(40-alb)<br>For albumin >40 g/L : = measured Ca – 0.02(Alb-40) |
| Magnesium | | 0.78 – 1.02 mmol/L |
| C Reactive Protein | | 0 – 8 mg/L |
| **Blood Gas**<br>PH<br><br>PO2<br><br><br>PCO2 | <br>0-1month<br>> 1month<br><br>0-1month<br>> 1 month<br><br>0-1 month<br>> 1 month | <br>7.33 – 7.49<br>7.35 – 7.46<br><br>60 – 76 mmHg<br>80 - 105 mmHg<br><br>27 – 40 mmHg<br>36 – 46 mmHg<br>(To convert to kPa = mmHg x 0.133) |
| Glucose | Neonate (term)<br>Neonate pre term<br>Fasting child | >1.6 mmol/L<br>>1.1 mmol/L<br>2.8 – 6.1 mmol/L |
| Lactate | | 0.7 – 2.1 mmol/L |

**Peripheral Infusion Site** – An injection site reaction is described as an uncommon reaction in the SPC (between 1 in 1000 patients and 1 in 100 patients). However, we are not aware of any studies in neonates. We will pilot measures to assess injection site reaction.

Tolerance to intravenous ciprofloxacin at the site of peripheral infusion will be monitored immediately before and after the infusion using a validated scale. The Infusions Standards by the Royal College of Nursing (UK) [45] recommend the Visual Infusion Phlebitis Scale developed by Jackson [46] the validation of which is unpublished but cited by Gallant and Schultz [47]. The scale of 0- 5 assesses phlebitis by monitoring pain, redness, swelling and palpable venous cord. The babies may receive other infusions of drugs or fluids through the same intravenous site therefore the site will be assessed using this scale immediately before the infusion and on completion of the 30 - 60 minute infusion and approximately 6 hours later (in-between the doses (appendix 5). Further information on other intravenous drugs administered by the peripheral venous line will be recorded with the pH. Data regarding the intravenous site such as removal of the cannula due to phlebitis or infiltration will be recorded and compared to the incidence neonates receiving other intravenous drugs.

Regulatory Compliance

This trial will be conducted in accord with the principles of the Helsinki Declaration and Good Clinical Practice according to the ICH document adopted in the European Union as http://www.ema.europa.eu/pdfs/human/ich/013595en.pdf (last accessed 25th January 2010) and applied to English law. Medicines for Human Use Clinical Trial Regulations (2004) 1031, as amended [48]. The trial will be conducted in accord with the opinion of the National Research Ethics Committee and NHS approval from Liverpool Women's NHS Foundation Trust including the Research and Development Manager and Clinical Director.

Regulatory Safety Reporting

All serious adverse events and non serious adverse events /reactions specified in the protocol whether or not they are attributable to the study intervention will be reported on case report forms. The reporting will commence when the patient has been consented and has become eligible for study monitoring. These will be reviewed by the investigators monthly to determine if there is reasonable suspected causal relationship to the intervention.

If there is evidence that there may be a novel causal relationship with the intervention, then the procedure for expedited reporting of suspected unexpected serious adverse reactions will be followed (section 7.4) All adverse events will be followed until satisfactory resolution or until the investigator responsible for the care of the participant deems the event to be chronic or the patient to be stable.

All expected Serious Adverse Events (SAE) will be recorded in the case report forms (CRF) but will not require expedited reporting [4] but will be reviewed by the DMC at regular intervals throughout the trial. The trial sponsor will collate and submit annual safety reports to MHRA and the appointed NRES committee. However, the MHRA, the trial sponsor and the chair of the MREC may also be informed about severe adverse events by the DMC chair if considered appropriate.

A standard reporting procedure will be provided on the reverse side of all SAE forms. A Trial Standard Operating Procedure with detailed information on reporting procedures will also be kept in site files at each 'trial station' in each centre. Copies of all correspondence relating to reporting will be maintained in the Investigator's files.

Regulatory adverse event or reaction reporting will commence once consent has been given and the baby has been determined as eligible to participate in the study and until the last study related procedure for that baby. (systematic data collection for non serious adverse events/reactions will continue until 3 days after the last dose).

*Pharmacovigilance Definitions:*

**Adverse event (AE)** -Any untoward medical occurrence in a subject recruited to a clinical trial, including occurrences which are not necessarily caused by or related with the treatment. An adverse event can be any unfavourable and unintended sign or symptom associated with the use of a medicinal product whether or not related to that product.

**Adverse reaction (AR)-**Any untoward and unintended response in a subject to an investigational medicinal product which is related to any dose administered to that subject.

**Serious adverse event (SAE),** serious adverse reaction (SAR) or unexpected serious adverse reaction (see 5.3)

Any adverse event, adverse reaction or unexpected adverse reaction, respectively, that

a) results in death,

b) is life-threatening,

c) requires hospitalisation or prolongation of existing hospitalisation,

d) results in persistent or significant disability or incapacity,

## Expected Serious Adverse Events NOT Requiring Expedited Reporting

Expected serious adverse events defined as follows will be recorded and reported but are exempt from expedited reporting to the sponsor and regulatory bodies as they are expected in this high risk populations [4].

Among extremely premature babies born at less than 34 weeks, the following are serious adverse events and incidence rates which could be reasonably expected for this group of babies during the course of the study.

| **Adverse event** | **Estimated Incidence < 28 weeks gestation** | **Estimated incidence 28 – 34 weeks gestation** |
|---|---|---|
| a) death | 20%* | 8%* |
| b) necrotising enterocolitis or focal intestinal perforation diagnosed on clinical grounds, or at surgery | 15%* | 3%* |
| c) intracranial abnormality (parenchymal haemorrhage or focal white matter damage) on cranial ultrasound | 15%* | 6%* |
| d) requirement for supplementary oxygen 28 days after birth | 55%* | 6%* |
| e) patent ductus arteriosus requiring medical or surgical management | 25%* | 8%* |

| | | |
|---|---|---|
| f) retinal surgery for retinopathy of prematurity | 5%* | 0.14* |
| g) pulmonary haemorrhage | 5%** | 0.5%** |
| h) persistent weight loss after 14 days after birth in the absence of infection. | 10%** | 1** |

* based on incidence of these complications at Liverpool Women's Hospital 1980 - 2004 (Prof. RWI. Cooke, personal communication, and Cooke RW[51])

** based on incidences estimated by the investigators

Based on data for those under 34 weeks the serious adverse events listed as expected will be **exempt from expedited reporting** for all babies in the study including those up to 52 weeks post menstrual age unless the Chief Investigator or DMC determine the incidence has increased.

*Suspected Unexpected Serious Adverse Reactions*

All serious adverse events will be reviewed by the Chief Investigator Dr. Mark Turner or another senior clinician nominated by the Sponsor. For each death or other serious adverse event, the CI, or his deputy, will make a judgment about whether the nature or severity of the circumstances surrounding the death is consistent with adverse events associated with the underlying conditions, or with ciprofloxacin (including literature cited in the protocol). In the event of a serious adverse event that has a causal relationship to ciprofloxacin and is not consistent with the information contained in this protocol or the Summary of Product Characteristics the event will be labelled as a Suspected Unexpected Serious Adverse Reaction (SUSAR). All events labelled as SUSARs will be reported to the sponsor within 24 hours of the decision being made. All SUSARs will be reported to the competent regulatory authority (MHRA) in accord with the Sponsor's Standard Operating Procedures.

*Data Monitoring Committee (DMC)*

An independent data monitoring committee has been established for this study:

Chairperson: Professor David Field, University of Leicester, UK.

Members: Professor Stephanie Laer, University of Düsseldorf, Germany.

Professor Neil Morton, University of Glasgow, UK.

The DMC will determine the frequency and the form in which they meet (personal meeting/ teleconference), which data they would like to see and how frequently. The investigators and Ethics Work Package of TINN will support the DMC to follow the DAMOCLES statement for DMC charters [49] [50]. The trial investigators will ensure that all the requested data is received

by the DMC during the trial. The DMC will assess safety aspects and inform the trial investigators who will then respond to their recommendations.

*Data Management*

The case report form (CRF) will not contain patient identifiable data. On recruitment each participant will be assigned a unique study number. The data in the CRF will be linked to clinical source documents by the unique study number. The tables linking unique study numbers to patients will be retained by the Research and Development Department at Liverpool Women's Hospital or Alder Hey Children's NHS Foundation Trust for 21 years to ensure data is available as the child develops and will not be passed on to other organizations. The investigators and institutions will permit trial-related monitoring, audits, REC review, and regulatory inspection(s). This will include direct access to source data/documents.

At LWH, the clinical source documents will be the electronic patient record contained within the unit's patient data management system. At the PICU the clinical source documents will be the written patient record and other aspects of the clinical record. The source document for PK assays will be the meditech system for blood results or nursing notes, patient charts and medical records.

*Quality Control and Quality Assurance*

The study will be monitored by LWFT R&D.

*Drug Product Quality Issues*

Ciprofloxacin will be stored and administered in accordance with the Marketing Authorisation Summary of Product Characteristics and Trust Drug Administration Protocol. The drug will be labelled during the infusion in accordance with Eudralex Annex 13 [51].

*Ethics*

The TINN Consortium includes Ethics and Safety work package co-ordinated by The University of Nottingham – Ethics Advisory Board (EAB) who will provide independent Ethics and Safety Monitoring of the program with European representatives who will liaise with the DMC. The PK study will be approved by the UK regulatory National Research Ethics Service.

The lack of benefit to participants does not preclude including procedures such as blood sampling episodes that are extra to clinical requirements (section 6.10). Some authors have written of the "Moral responsibility to attain thorough pediatric drug labelling" [52].

*Statistical Methods*

Pharmacokinetic data will be made available by clinical investigation center (CIC, Inserm 9202, Hôpital Robert Debré) on a standard Microsoft Office Excel spreadsheet For subsequent modeling using NONMEM version 6.2 (Globomax, USA).

A detailed statistical analysis plan will be developed. The principles underlying this plan are as follows:

Model selection criteria will include: (i) successful minimisation, (ii) standard error of estimates, (iii) number of significant digits, (iv) termination of the covariance step, (v) correlation between model parameters and (vi) acceptable gradients at the last iteration.

Continuous and categorical covariates will be tested during the primary analysis. For univariate analyses, additional parameters leading to a decrease in the objective function of 3.84 will be considered significant ($p<0.05$). During the final steps of the model building, only the covariates which resulted in a difference of objective function $\geq 7.88$ ($p<0.005$) will be kept in the final model.

Validation of the final model will be based on graphical and statistical methods. A suitable model is defined as including approximately 90% of data points within the 5th-95th prediction interval (approximately 5% above 95th and 5% below 5th). The final model will also be assessed by visual predictive check (VPC) and metric normalized prediction distribution errors (NPDE). The following graphs will be plotted by NPDE R package: (i) QQ-plot of the NPDE; (ii) histogram of the NPDE; (iii) NPDE versus time and (iv) NPDE versus predicted concentrations. The NPDE is expected to follow the N (0, 1) distribution.

Optimal dosing regimens PK parameters and their variability estimates from the preliminary model will be used to define the optimal dosing regimens to be used in neonates and infants. The optimised dosing regimen will take consideration of the PK-PD relationship published in adults, indicating that the AUC/MIC should be > 100. Hence, the model will be used to simulate various dosing regimens in various kinds of children (i.e. according to covariates), provided they allow attaining this PK-PD endpoint for the most relevant bacterial species concerned by this treatment (mean MIC for each strain). A secondary model with covariates will also be tested.

The optimised dosage regimen will be evaluated in subsequent PK studies (particularly as a component of the TINN registry study).

Safety outcomes will be tabulated. No statistical analysis will be performed on safety data.

*Financing and Insurance*

**Funding:** The study is funded by TINN European Research Network (collaborative Project) supported by the European Commission under the Health Co-operation Work Program of the 7th Framework Program, project .

**Financial disclaimer** - The drug products used for the PK study are generic products currently purchased through the NHS pharmacy.

The TINN consortium will liaise with the pharmaceutical industry regarding improved ciprofloxacin formulations for future work packages.

The TINN consortium includes a pharmaceutical small-medium-sized enterprise (SME), EPMC. (http://www.epmc-pharma.com/index.html) EPMC may become the PUMA holder or may license the PUMA to a different company. None of the investigators have any financial interest relating to EPMC.

*Sponsorship Arrangements*

The trial will have two co-sponsors: University of Liverpool and Liverpool Women's NHS Foundation Trust (LWFT). There will be a written agreement about the division of sponsorship responsibilities between these two organizations. In brief the University of Liverpool will be responsible for funding the trial (based on its contract with the European Commission as a participant in TINN). LWFT will undertake all other sponsorship responsibilities (this will be funded by a subcontract between the University of Liverpool and LWFT).

*Insurance*

Cover for negligent harm arising from trial-related activities conducted on NHS premises by staff with substantive or honorary NHS contracts will be provided by the NHS Litigation Authority (NHSLA) since the trial will be co-sponsored by Liverpool Women's NHS Foundation Trust. Indemnity for Dr. Turner's actions as an employee of the University of Liverpool will be provided by the University.

***Publication Policy***

This study will be published in accord with TINN 'Treat Infection in Neonates' policy. The study aims to licensing ciprofloxacin for neonates by contributing to a specific neonatal Marketing Authorisation therefore will be required to report all data to the EMEA. The study has been registered on the EUDRACT data base EudraCT: 2010-019955-23. For the primary published report of the study, the first author will be Hill and the last author will be Turner.

All data fields on EudraCT will be completed (including protocol and results fields) as indicated in the most recent guidance [3]. Raw data from the blood and CSF PK samples will be made available to other investigators subject to the agreement of the TINN Executive and the EC.

[3] http://ec.europa.eu/health/files/eudralex/vol-10/2009_02_04_guidelines_paed_en.pdf (last accessed 28th August 2010).

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Appendix 1 PK Studies Ciprofloxacin

| N' | Publication | N | Dosing schedule | Duration of infusion | Time of Cmax | Cmax | Trough level | Plasma half-life | Evidence of accumulation | Volume of distribution | Plasma protein binding | CSF concentration | Excretion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Chapter 29 of Antibiotic and Chemotherapy, Finch et al. 2003<br>[ADULTS] | n.r. | 200mg IV | 15 minutes | End of infusion | 3.5mg/L | n.r. | 3 – 4h | *n.r. | 3 – 4 L/kg | 20-40% | 50% of simultaneous plasma values | 75% of IV dose appear in the urine over 24 h |
| **[6]** | Lipman<br>[1 – 5 years old] | 10 | 10mg/kg/dose twice daily | 1 hour | End of infusion | 7.38 (1.35) d0, 7.78 (1.56) d2, 6.38 (1.35) d7 | 0.14 (2.03) d0, 0.21 (2.75) d2, 0.10 (1.74) d7 | 2.84 (1.18) d0, 3.13 (1.41) d2, 2.82 (1.18) d7 | No | 1.44 (1.17) d0,<br>1.43 (1.19) d2, 1.76 (1.23) d7 | *n.r. | *n.r. | *n.r. |
| **[6]** | Lipman<br>[INFANTS] | 10 | 10mg/kg/dose twice daily | 1 hour | End of infusion | 6.08 (1.23) day 0, 9.03 (1.85) day 2, 5.81 (1.35) day 7 | 0.21 (2.39) day 0, 0.21 (2.77) day 2, 0.16 (1.86) day 7 | 3.67 (1.51) day 0, 3.32 (1.90) day 2, 4.23 (1.32) day 7 | No | 2.06 (1.33) day 0, 1.49 (1.53) day 2, 2.05 (1.32) day 7 | *n.r. | *n.r. | *n.r. |
| **[21]** | Bannon | 6 | 5 mg/kg/day twice daily | *n.r. | 30minutes after IV injection | 3.1 mg/L (range 1.45 to 5.7 mg/L) after the third dose | 1.25 mg/L (range 0.04 to 2.6 mg/L). | *n.r. | *n.r. | *n.r. | *n.r. | 64% of simultaneous serum values | *n.r. |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **[32]** | Aggarwal et al., 2004 | 24 | 10mg/kg/dose twice daily | 30 minutes | 15 minutes after dose | 2.3 mg/L (range 0.2 to 6.8 mg/L) day 1<br><br>3.0 mg/L (range 0.1 to 7.1 mg/L) day 3<br><br>2.7 mg/L (range 0.5 to 7.1 mg/L) day 7 | 0.7 mg/L (range 0 to 2.1 mg/L) day 1<br><br>0.8 mg/L (range 0 to 2.1 mg/L) day 3<br><br>1.0 mg/L (range 0.1 to 3.5 mg/L) day 7 | *n.r. | No | *n.r. | *n.r. | *n.r. | *n.r. |
| **[33]** | Goepp, et al | 1 infant with ventriculitis | 35 mg/kg/day twice daily | 2 hours | 15 minutes after end of infusion | 11.6 mg/L | 0.9 mg/L | 2.89h for eighth dose<br><br>3.79 h for twenty-fourth dose | *nr | *nr | *nr | 12.1% -175% of simultaneous serum values | |
| **[34]** | Wessalowski et al. | 1 infant with brain abscess | *nr | | *nr | *nr | 0.1 to 2.8 mg/L | | | | | | |
| **[35]** | Van den Oever et al. | 1 infant | 20 mg/kg/day twice daily | 30 minutes | *nr | *nr | *nr | *nr | *nr | *nr | *nr | *nr | *nr |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **[53]** | Payen et al.<br><br>Some were cystic fibrosis patients. These data were excluded from our review | -53 pediatric patients<br>aged 1 day to 17 years and 2 young adults<br>- 3 neonates | Neonate: 5 to 17 mg/kg | 30 min | *nr | *nr | *nr | Mean: 16.6<br>Min: 10.5<br>Max: 24.5 | *nr | Mean: 7.19<br>Min: 3.8”<br>Max: 12.4 | *nr | *nr | *nr |

n.r. not reported

## SUMMARY OF PRODUCT CHARACTERISTICS

### 1 NAME OF THE MEDICINAL PRODUCT

Ciprofloxacin 2mg/ml, Solution for Infusion

### 2 QUALITATIVE AND QUANTITATIVE COMPOSITION

Each presentation of Ciprofloxacin 2mg/ml infusion contains the following:

**Ciprofloxacin 100 mg/50 ml, Solution for Infusion**

1 ml of Solution for Infusion contains 2mg Ciprofloxacin as 2.544mg Ciprofloxacin lactate.

Each 50 ml vial contains 100 mg Ciprofloxacin (as Ciprofloxacin Lactate).

Excipient: Each 50ml contains 7.7 mmol (177 mg) Sodium.

**Ciprofloxacin 200 mg/100 ml, Solution for Infusion**

1 ml of Solution for Infusion contains 2mg Ciprofloxacin as 2.544mg Ciprofloxacin lactate

Each 100 ml vial contains 200 mg Ciprofloxacin. (as Ciprofloxacin Lactate).

Excipient: Each 100ml contains 15.4 mmol (354 mg) Sodium.

**Ciprofloxacin 400 mg/200 ml, Solution for Infusion**

1 ml of Solution for Infusion contains 2mg Ciprofloxacin as 2.544mg Ciprofloxacin lactate.

Each 200 ml bottle contains 400 mg Ciprofloxacin. (as Ciprofloxacin Lactate).

Excipient: Each 200 ml contains 30.8 mmol (708 mg) Sodium.

For a full list of excipients see section 6.1

### 3 PHARMACEUTICAL FORM

Solution for infusion

Clear, colourless to slightly yellow solution.

### 4 CLINICAL PARTICULARS

#### 4.1 Therapeutic indications

Ciprofloxacin is indicated for the treatment of serious and/or life-threatening infections caused by ciprofloxacin-susceptible pathogens. The following indications can be considered for treatment with Ciprofloxacin when oral therapy is not possible or not reliable:

- complicated urinary tract infections
- infections of the lower respiratory tract including pneumonia caused by aerobic gram-negative bacteria, in case of Streptococcus pneumoniae infections ciprofloxacin is not the substance of first choice.
- complicated skin and soft tissue infections
- osteomyelitis

Children and adolescents:

Ciprofloxacin may be used for 2nd and 3rd line treatment of complicated urinary tract infections and pyelonephritis in children and adolescents of 1-17 years of age and for the treatment of acute pulmonary exacerbation of cystic fibrosis associated with Pseudomonas aeruginosa in children and adolescents of 5-17 years of age. The use of

ciprofloxacin in paediatric patients with complicated urinary tract infections and pyelonephritis should be restricted to infections caused by organisms for which ciprofloxacin is the drug of choice, based on the results of antimicrobial susceptibility testing. Treatment should be initiated by a physician who is experienced in the treatment of severe infections in children and adolescents and after careful benefit/risk evaluation, due to possible adverse events related to joints and / or surrounding tissues (see sections 4.4 and 5.1)

In case of mixed infections with anaerobes ciprofloxacin must be combined with other antibiotics effective against anaerobes.

Consideration should be given to official guidance on the appropriate use of antibacterial agents.

**4.2 Posology and method of administration**

The solution for infusion should be administered over an infusion period of 60 minutes.

Due to the increased risk of local reactions, higher intravenous doses in particular should only be administered via a large vein or a central line. Mixing with other solutions: see sections 6.2 and 6.6.

The duration of treatment depends upon the severity of infection, clinical response and bacteriological findings. Generally, acute and chronic infections (e.g. osteomyelitis and prostatitis, etc), where the causative organism is known to be sensitive to ciprofloxacin, should be treated for at least three days after the signs and symptoms of the infection have disappeared.

Adults:

The adult dosage is 200 – 400 mg ciprofloxacin twice daily.

In case of very serious, life-threatening or recurrent infections the dosage can be increased to 400 mg three times daily. The maximum daily dose is 1200 mg.

Osteomyelitis:

Prior to initiation of therapy, bacteriological sensitivity tests should be conducted. As with all other antibiotics, the patient should be monitored during therapy for the development of resistant strains of initially sensitive bacteria, especially P. aeruginosa and S. aureus (see the relevant statements in section 5.1). Average duration of treatment can be 4-6 weeks. If a prolonged treatment is necessary, a reassessment of treatment should be done at 2 months at the latest.

Impaired renal function:

In patients with a creatinine clearance in the range 31 – 60 ml/minute/1.73 $m_2$ or a serum creatinine concentration in the range 124 – 174 µmol/l, the maximum daily intravenous dose is 800 mg.

If creatinine clearance is ≤ 30 ml/minute/1.73 $m_2$ or the serum creatinine concentration is ≥ 175 µmol/l, the maximum daily intravenous dose is 400 mg.

In patients on haemodialysis or CAPD, the maximum daily intravenous dose is also 400 mg. On the dialysis days, the dose is given after the haemodialysis session.

Impaired hepatic function:

In case of impaired hepatic function it is not necessary to adjust the dosage.

Impaired renal and hepatic function:

Dose adjustment according to renal function. Monitoring the level of active substance in the blood provides the most reliable basis for dose adjustment.

Elderly:

Due to the higher plasma levels in the elderly it is advisable to administer doses based on creatinine clearance and severity of disease.

Paediatric patients:

Acute lower respiratory tract infections caused by Pseudomonas aeruginosa in children and adolescents (5-17years) with cystic fibrosis: Twice daily intravenous administration of 15 mg/kg bodyweight, or 10 mg/kg bodyweight three times daily (maximum of 1200 mg per day).

Complicated urinary tract infections and pyelonephritis:

For complicated urinary tract infections or pyelonephritis the dose is 6 to 10 mg/kg IV every 8 hours with a maximum of 400 mg per dose or 10 to 20 mg/kg orally every 12 hours with a maximum of 750 mg per dose.

For complicated urinary tract infections or pyelonephritis the duration of treatment is 10-21 days.

The dosage in children with impaired renal and/or hepatic function has not been investigated.

**4.3 Contraindications**

Ciprofloxacin is contraindicated in**:**

- patients with a hypersensitivity to ciprofloxacin, chinolin carboxylic acid derivatives or to any of the excipients
- children under 5 years of age. With regard to the safety and use of ciprofloxacin in children, see also section 4.4
- Children and growing adolescents except for the treatment of acute pulmonary exacerbations of cystic fibrosis in children aged 5 to 17 years.
- pregnancy and lactation
- patients with a history of tendon disorder related to fluoroquinolone administration
- Concurrent administration of ciprofloxacin and tizanidine

**4.4 Special warnings and precautions for use**

Renal and urinary system:

Crystalluria related to the use of ciprofloxacin has been reported. Patients receiving ciprofloxacin should be well hydrated and excessive alkalinity of the urine should be avoided.

Hepatic disorders:

Cases of hepatic necrosis up to life threatening hepatic failure have been reported with ciprofloxacin (see section 4.8). In the event of any signs and symptoms of hepatic disease (such as anorexia, jaundice, dark urine, pruritus or tender abdomen), treatment should be discontinued.

Blood and lymphatic system:

Patients with a family history of or actual defects in glucose-6-phosphate dehydrogenase activity are prone to haemolytic reactions with quinolones, and so ciprofloxacin should be used with caution in these patients.

Central nervous system:

As with other fluoroquinolones, specific undesirable effects with regard to the central nervous system must be taken into account when using Ciprofloxacin . In patients with epilepsy or other lesions of the central nervous system (e.g. reduced convulsion threshold, a history of epileptic seizures, diminished cerebral blood flow, changes in brain structure or stroke), ciprofloxacin is only to be used after carefully weighing the benefits against the risk, because the possibility of central nervous side effects puts these patients at increased risk.

Anaphylactic/anaphylactoid reactions can in very rare cases develop into life-threatening shock, sometimes even after the first administration of ciprofloxacin. In that case, the ciprofloxacin treatment must be discontinued, and medical treatment for shock should be given.
Local reaction:
Local reactions have been reported after intravenous administration of ciprofloxacin. These reactions occur more frequently when the infusion time is 30 minutes or less. These may be manifested as local skin reactions, which rapidly disappear after the infusion has been completed.
Further intravenous administration is not contraindicated unless the reactions reoccur or worsen.
Because ciprofloxacin has some activity against Mycobacerium tuberculosis, false-negative cultures may occur when the specimens are obtained during ciprofloxacin treatment.
Ciprofloxacin contains 15.4 mmol (354 mg) sodium per 100 ml solution for infusion. This has to be taken into consideration for patients on a controlled sodium diet.

The undesirable effects sometimes occur already after the first administration of ciprofloxacin. Depression or psychoses lead to self-endangering behaviour in some cases. If such reactions occur, treatment with ciprofloxacin must be discontinued immediately and the treating physician informed.
Cardiac disorders:
Since ciprofloxacin is associated with very rare cases of QT prolongation (see section 4.8) caution should be exercised when treating patients at risk for torsade de pointes arrhythmia.
Pediatric use:
Ciprofloxacin has been shown to cause arthropathy in weight-bearing joints of immature animals. Safety data from a randomized double blind study on ciprofloxacin use in children (Ciprofloxacin: n=335, mean age = 6.3 years; comparators: n=349, mean age = 6.2 years; age range = 1 to 17 years) revealed an incidence of suspected drug related arthropathy (discerned from joint-related clinical signs and symptoms) by Day +42 of 7.2% and 4.6%. Respectively, an incidence of drug-related arthropathy by 1-year follow-up was 9.0% and 5.7%. The increase of suspected drug related arthropathy over the time was not statistically significant between groups. Treatment should only be initiated after a careful benefit/risk evaluation, due to possible adverse events related to joints and/or surrounding tissue.
The use of ciprofloxacin for indications other than the treatment of acute pulmonary exacerbation of cystic fibrosis caused by P. aeruginosa infection (children aged 5 – 17 years), complicated urinary tract infections and pyelonephritis (children aged 1 – 17 years) and for the use in inhalational anthrax (post-exposure) has not been evaluated in clinical trials and the clinical experience is limited. The use of ciprofloxacin should follow the official guidance.
Gastrointestinal tract:
When during or after the treatment with ciprofloxacin or another fluoroquinolone severe and persistent diarrhoea occurs, pseudomembranous colitis must be taken into account (life-threatening with possibly fatal outcome). In that case the ciprofloxacin therapy must immediately be discontinued and an appropriate treatment initiated. Antiperistaltics are contraindicated. The transaminase or alkaline phosphatase concentrations may

temporarily increase or cholestatic icterus might occur, especially in patients with previous liver damage.

Musculoskeletal system:

If there is any indication of tendinitis (e.g. painful swelling) the administration of ciprofloxacin or other fluoroquinolones must immediately be discontinued, the affected extremity should not be strained and a physician must be consulted. Very rarely, a partial or total rupture (in particular of the Achilles tendon) has been reported, especially in elderly patients who were previously treated systemically with glucocorticoids.

Ciprofloxacin may cause an exacerbation of Myastenia gravis symptoms. Therefore, in case of any symptom indicating an exacerbation of Myastenia gravis a physician must be consulted.

Photosensitivity:

Ciprofloxacin and other fluoroquinolones may cause photosensitivity. Therefore, it is recommended to avoid prolonged exposure to sunlight or UV light during treatment with ciprofloxacin. However, if this is not possible the patient is recommended to use a sun-protection cream. When photosensitivity occurs the treatment must be discontinued.

Hypersensitivity:

Hypersensitivity reactions and allergic reactions occurred in some cases after the first administration of ciprofloxacin. If such reactions occur, a physician must immediately be consulted.

**4.5 Interaction with other medicinal products and other forms of interaction**

Probenecid

Probenecid inhibits the renal excretion of ciprofloxacin resulting in an increase in the plasma concentration of ciprofloxacin.

CYP1A2

Ciprofloxacin inhibits CYP1A2 and thus may cause increased serum concentration of concomitantly administered substances metabolised by this enzyme (e.g. theophylline, clozapine, tacrine, ropinirol, tizanidine). Therefore, patients taking these substances concomitantly with ciprofloxacin should be monitored closely for clinical signs of overdose. Determination of serum concentrations, especially of theophylline, and dose adjustments may be necessary. The interaction between theophylline and ciprofloxacin is potentially life-threatening.

Other xanthine derivatives

On concurrent administration of ciprofloxacin and caffeine or pentoxifylline (oxpentifylline), raised serum concentrations of these xanthine derivatives were reported.

Phenytoin

Simultaneous administration of ciprofloxacin and phenytoin may result in increased or reduced serum levels of phenytoin such that monitoring of drug levels is recommended.

Methotrexate

Renal tubular transport of methotrexate may be inhibited by concomitant administration of ciprofloxacin potentially leading to increased plasma levels of methotrexate. This may increase the risk of methotrexate associated toxic reactions. Therefore, patients receiving methotrexate therapy should be carefully monitored when concomitant ciprofloxacin therapy is indicated.

Ciclosporin

Following concomitant administration of ciprofloxacin and ciclosporin a transient increase of the serum creatinine concentration has been observed in separate cases. Therefore, the serum creatinine concentration must be checked regularly (twice per week) in these patients.

Oral anticoagulants (e.g. warfarin)

Ciprofloxacin, like other quinolones, may enhance the effect of coumarin derivates including warfarin. In the case of concomitant administration of these products, prothrombin time (PT) or other suitable coagulation tests should be monitored. If necessary, the oral anticoagulant dose should be adjusted as appropriate.

Glibenclamide

When used simultaneously, ciprofloxacin may, in certain cases, increase the effect of glibenclamide (hypoglycaemia).

NSAIDs

Animal trials have shown that the concurrent administration of very high doses of fluoroquinolones and certain NSAIDs (but not acetylsalicylic acid) may provoke convulsions.

Mexiletine

Simultaneous administration of ciprofloxacin and mexiletine can lead to increased plasma concentrations of mexiletine.

**4.6 Pregnancy and lactation**

Pregnancy

Use during pregnancy is contraindicated. There are limited data on the use of ciprofloxacin during pregnancy. Up to now, no evidence has been found of an increased risk of congenital abnormalities or other undesirable effects following use of ciprofloxacin or other quinolones during the first trimester. Teratogenic effects have not been observed in animal experimental research. In juvenile and prenatal animals exposed to quinolones effects on immature cartilage have been observed. Since the risks for humans are unknown Ciprofloxacin must not be administered during pregnancy (see section 4.3).

Lactation

Ciprofloxacin is excreted in breast milk. Due to the risk of arthropathy and other potentially severe toxicity in the infant, ciprofloxacin is contraindicated during lactation (see section 4.3).

**4.7 Effects on ability to drive and use machines**

Ciprofloxacin has minor or moderate influence on the ability to drive and use machines. When undesirable effects on the central nervous system, like dizziness, occur, it is prohibited to drive a vehicle or to operate machines.

**4.8 Undesirable effects**

Adverse reactions have been reported in 5-14% of patients receiving ciprofloxacin. Most frequent adverse reactions involve the gastro-intestinal tract and the central nervous system.

The following adverse reactions have been observed:

| In this section undesirable effects are defined as follows: | (≥1/10) very common |
|---|---|
| common | (≥1/100 to <1/10) |
| uncommon | (≥1/1,000 to <1/100) |
| rare | (≥1/10,000 to <1/1,000) |
| very rare | (<1/10,000), not known (cannot be estimated from the available data) |

Alder Hey Children's NHS
NHS Foundation Trust

Liverpool Women's NHS
NHS Foundation Trust

# Information Sheet for Parents/Guardians

## A study about the antibiotic 'Ciprofloxacin' used to prevent or treat infection in neonates and young infants.

We would like to invite you to allow your baby to participate in a research study. Take enough time to decide whether or not you wish to take part. It is important for you to understand why the research is being done and what it will involve before deciding. Please read the following information carefully and discuss it with others if you wish, or ask us if you would like more information.

**The purpose of this study is to monitor the effects of Ciprofloxacin an antibiotic.** This drug has been used in babies for a long time. It has been tested extensively in adults and children but less so in neonates. By monitoring babies who are prescribed this medicine as part of their clinical care will help us to work out the most effective dose to treat infections, the time to wait between dose and which, if any, side effects are important.

**Why has my baby been chosen?**

You have been asked to consider taking part as your baby is less than 3 months of age and during the time they are in hospital they may require the antibiotic 'Ciprofloxacin' to prevent or treat infection. Your baby's doctor (not the research team) may make a decision to start this antibiotic (it would be given through a drip as part of their clinical care). We would like to monitor babies as soon as the treatment is started therefore would like to ask for your consent at this stage.

We would like to include 50 babies for this study. Some of the babies will be treated at Liverpool Women's Hospital and some will be treated at Alder Hey Children's Hospital.

**Does my baby have to take part?**

No - It is up to you to decide whether your baby can take part and which part of the study you would be willing to consent to. This decision will not affect the care your baby receives. If you do decide to take part then change your mind you are free to withdraw at any time without giving a reason. You may request that any information already collected can also be withdrawn.

**What will happen to my baby if s/he takes part in the drug level and safety study?**

- The research team will collect details from the baby's medical notes and complete a physical assessment.
- To monitor the level of the antibiotic in the baby's blood we will collect 2-3 blood samples (0.2 ml approximately 4 – 6 drops for each sample or less in very small babies) on the first and last day of treatment.
- A sample of faeces from the baby's nappy will be collected whilst in hospital and between 4-6 weeks later (may be posted from home if no longer in hospital).
- We will monitor your baby carefully for any potential side effects to the antibiotic.

**Additional consent for further samples:**

- **CSF Sample -** in some situations when a baby shows signs of infection their doctor may decide to test a sample of the fluid that surrounds the brain and spinal cord 'CSF' (cerebrospinal fluid) to see if they need to treat infection in this area. If your baby needs this test for their clinical care we would like to use a few extra drops (0.5ml) to see if the antibiotic is effective in treating infection in this part of the body.
- **'Genetics**' –some babies may respond better to this antibiotic than others, this could be because different genes have a better response than others. We would like to asses this by analysing DNA obtained from blood already taken for your baby's care or by wiping a cotton bud in the baby's mouth. The genetic sample will only be used for the purpose of collecting information on babies, response to infection or this medicine.

If you choose to consent to the study you will be asked to sign the appropriate boxes on the consent form stating which samples you have agreed to.

**What are the possible benefits of taking part?**

There are no direct benefits to your baby but the study may help the clinical team to know more about the best way to treat babies in the future. Many studies of different treatments over the years have enabled us to develop the care of babies.

Studies involving children only provide expenses to families if they are required to travel, therefore there will be no financial compensation.

**Are there any side effects?**

The study involves closely monitoring the effects of ciprofloxacin therefore there are no side effects to the monitoring. Ciprofloxacin would be given as part of the clinical care not as part of the study. This study will check in more detail the effects of this drug by closely observing the effects of this treatment.

**What are the disadvantages or risks of this procedure when taking part?**

Taking blood samples may lead to some discomfort for the baby. Blood samples will be taken by staff experienced in taking blood from premature or new born babies. The team will make an effort to minimise any distress with involvement of parents.

When babies require this antibiotic they often require other blood samples for their clinical care, whenever possible we will plan to take these at the same time as the study sample to minimise disturbing your baby. Some babies will already have a line into a blood vessel to give fluid, other medicines or for monitoring, when possible we will aim to take the sample from this line. In some cases when this is not possible then we may take a small amount of blood from a vein or a heel prick. If we are unable to take the sample or if the baby is too distressed then we will stop.

**What happens after the study?**

With your permission the researcher may contact you to ask a few questions about your baby at a later stage. Also, if you would like to receive a summary of the results of the study this will be sent to you. (If you agree you will be asked to initial the appropriate box on the consent form).

**What if something concerns me?**

If you have any concern about the study you may speak to the researcher or the baby's doctor or nurse who will discuss this with you and try to provide an answer. If you wish to complain, or have concerns about any aspect of the study the National Health Service complaints mechanisms may be followed.

**Will my taking part in this study be kept confidential?**

If you do consent to the research, your baby's medical records may be read by the research team or inspected by regulatory authorities to check that the study is being carried out correctly. With your permission we will write to your GP to let them know that your baby has been part of the study (this is routine for any research). Your baby's name or other personal details will not be given to anyone else outside the hospital. All information will be stored confidentially.

If you have agreed to a DNA sample this will be given a code so that it is confidential then stored in the TINN Genetics Laboratory in European for a maximum of 10 years.

**What will happen to the results of the research study?**

The results will contribute to the information on the use of medicines for children and may be used to guide the dosage and administration of this drug specifically for neonates. We will publish the results of the study in medical journals, present to other relevant staff and put a summary of the results onto the hospital web site. The results will not be available until after the research has finished. We plan to get a license for this medicine in newborn babies. If you would like us to send you a summary of the results please initial the appropriate section on the consent form.

**Who is organising and funding this research?**

The study is a European research network project supported by the European Commission (Health Co-operation Work Programme of the 7th Framework Programme).

**Who has reviewed the study other than the research team?**

The study has been reviewed by the UK National Research Ethics Service, the Medicines for Children Research Network including nurses and doctors who are experts in the care of new born babies and by the Research Governance Group at Liverpool Women's Hospital and Alder Hey Children's NHS Foundation Trust.

**Contact for further information?** Dr Mark Turner Consultant Neonatologist 0151 708 9988 or 24 hour access via switch board Email: mark.turner@liverpool.ac.uk

Helen Hill Research Fellow 0151 702 4111 Email: Hhill@liv.ac.uk Email: TINN@lwh.nhs.uk

The clinical team support this study, including the Neonatal Consultants or Consultants at the Childrens's Hospital.

*Thank you for reading this information sheet.* Version number1 EudraCT number 2010-019955-23

Alder Hey Children's NHS NHS Foundation Trust  Liverpool Women's NHS NHS Foundation Trust

# PARENTAL CONSENT FORM

To study the antibiotic Ciprofloxacin used to prevent or treat infection

in neonates and young infants **Researchers: Chief Investigator Dr Mark Turner, Research Fellow Helen Hill**

**Name of baby……**

**Please Initial**

1. I confirm that I have read and understood the **information sheet** and have had the opportunity to ask questions and these have been answered satisfactorily. ☐
2. I understand that my baby's **participation is voluntary** and that we are free to withdraw at any time, without giving reason, without my babies medical care or legal rights being affected. ☐
3. I understand that sections of any of my baby's medical notes may be looked at by the research team or the regulatory authorities for this research. I give permission for these individuals to have **access to my baby's records.** ☐
4. I understand that my **family doctor will be informed** that my child is taking part in this study**.** ☐
5. **I agree for my baby to take part in the ciprofloxacin safety and drug level study** ☐
6. **I agree for my baby's DNA to be analysed for the genetic part of this study**
7. **I agree that if my baby's doctor decides to test cerebrospinal fluid they may take an additional sample at the same time for this study.** ☐
8. I would like a **summary of the results** of the study when the research is completed**.** ☐

**9**. I agree to be **contacted at a later** stage regarding the study. ☐

**Name of parent:……  Relationship to baby……**

**Signature…….  Date……**

**Researcher……Signature ……Date:……**

*Photocopy and give 1 copy to the parent, 1 for research file, original in the medical notes*

EudraCT number 2010-019955-23 **Patient's study number**: **Version number 1**

## Leaflet /Flyer

Tinn

**Treat Infections in Neonates**

**INFORMATION FOR PARENTS OF BABIES UNDER 3 MONTHS AGE**

# Ciprofloxacin Study

**for babies less than 3 months age at**

**Liverpool Women's Hospital**

**&**

**Alder Hey Children's Hospital**

Ciprofloxacin, it is an 'antibiotic' used to treat or prevent infection in neonates and young infants. It is often used when the infection has not responded to other antibiotics

We would like to ask your permission for your baby to take part in the study. Then if at any time during their hospital stay they are prescribed this medicine as part of their care the research team will be able to monitor the baby as soon as they start the treatment.

The antibiotic has been used for many years in newborn babies and infants. We believe ciprofloxacin is a safe medicine in newborn babies.

The study will allow us to update prescribing guidelines by checking the most effective dose to treat infections and the time we should wait between doses.

By collecting details from the baby's medical records, drug levels from blood tests, stool sample from the nappy and by completing a physical assessment (as the clinical team do routinely) we can monitor possible side-effects very carefully.

We would like to give you an information sheet to explain this in more detail then allow you to ask questions about the study.

This information will be offered to families of babies who are in hospital and under 3 months of age.

If you then choose to take part you will be asked to sign a consent form, this will allow us to monitor your baby if they are prescribed ciprofloxacin for their clinical care.

**Contact details:**

If you have any questions at any time the doctors, nurses and researchers will be pleased to provide more information. Please contact us or ask any of the clinical staff to contact us on your behalf:

**Liverpool Women's NHS Foundation Trust**
Dr Mark Turner, Consultant Neonatologist Tel: 0151 702 4029 (Secretary)
0151 702 4193 (Neonatal Unit)
Helen Hill Research Fellow
Tel: 0151 702 4111
**Alder Hey Children's NHS Foundation Trust**
Dr Sarah Mahoney
Consultant Intensivist
Alder Hey NHS Foundation Trust
Tel: 0151 228 4811
Email: TINN@lwh.nhs.uk

Alder Hey Children's NHS Foundation Trust

NHS National Institute for Health Research


# Information for Parents of all Babies under 3 months of Age

We are leading a European study about an antibiotic called **Ciprofloxacin** used to treat infection in babies.
Many studies of different treatments over the years have enabled us to develop the care of babies.

**What is Ciprofloxacin?** It is an antibiotic we use to treat infections. It is often used when the infection has not responded to other antibiotics. Ciprofloxacin has been used for many years in new born babies and infants.

**Why are we studying this medicine?** By monitoring babies who are prescribed this medicine will help us to work out the most effective dose to treat infections, the time to wait between doses and which, if any, side effects are important.

**We would like to provide information to families about the study** then allow you time to ask questions and consider whether your baby may take part in the monitoring. The medicine would be prescribed by your baby's doctor as part of your baby's clinical care not as part of the research. The researchers would like to ask for your consent so that if your baby needs this medicine at any time we may monitor them as soon as the treatment is started.

Please contact us: Tel: 0151 702 4193
**Alder Hey Hospital:** Dr Sarah Mahoney Consultant Paediatrician Tel: 0151 228 4811
Dr Stephane Paulus Consultant Paediatrician Tel: 0151 228 4811
**Both sites:** Helen Hill Research Fellow 0151 702 4111
**Email** TINN@lwh.nhs.uk.

Liverpool Women's NHS
NHS Foundation Trust

Alder Hey Children's NHS
NHS Foundation Trust

# TINN TREAT INFECTIONS IN NEONATES

**Appendix II**

**Pharmacokinetics, tolerability and short-term safety of ciprofloxacin in neonates with suspected (or proven) Gram Negative infection**

# Case Report Form

1st letter of patient name: |__|

1st letter of patient surname: |__|

Center Number: |__|__| (centre LWH 1 AHH 2)

Inclusion number in the study: |__|__|__|

Date of Inclusion

Date of Birth: |__|__|/|__|__|/|__|__|__|__| (dd/mm/yyyy)

**INCLUSION CRITERIA**

| | Yes | No |
|---|---|---|
| Information Leaflet Provided to Parents | | |
| 24– 52 weeks post menstrual age | | |
| Receiving ciprofloxacin as part of clinical care | | |
| Parental consent | | |

**EXCLUSION CRITERIA**

| | Yes | No |
|---|---|---|
| Likely not to survive 48 hours in the judgement of attending physician | | |
| Ciprofloxacin administered before day 5 of life | | |
| ECMO or Haemofiltration | | |

**Person(s) with parental responsibility has consented to:**

**Cerebrospinal fluid** ☐

**DNA sample** ☐

**Contacted by Study Team at a later stage** ☐

**Request a copy of the study results** ☐

**Consent form signed by the parents (or legal representative):**
**(According to the local law, at least 1 parent has to sign consent in the UK. Both parents have to sign the consent in France)**
**Date** |__|__|/|__|__|/|__|__|__|__| (dd/mm/yyyy)**:**

| | |
|---|---|
| 1 copy given to parents | Yes☐ |
| 1 copy case report form | Yes☐ |
| 1 copy medical notes | Yes☐ |

**Eligibility:**
**Patient commenced ciprofloxacin and is now eligible signed…… (medically qualified) Date……..**
**Record eligibility in is determined by a medically qualified)** ☐

**Demographics**

**Gender:** ☐ Male ☐ Female

**Maternal race**

☐Caucasian ☐ African ☐ Asian ☐ other (specify):

**Post menstrual age at birth:** ___ ___ weeks ___ ___ days

**Post menstrual age when starting study weeks ……..days…….**

**Birth weight:** ___ ___ g

**Fetal growth restriction*** ☐ yes ☐ no

***According to the local curve:**

**In UK, birth weight below 9$^{TH}$ CENTILE**

**In France: Audipog (**http://www.audipog.net/crois-foet.php**)**

**Apgar score at 5 minutes -------**

**Surfactant:** ☐ yes ☐ no

**Indication of ciprofloxacin treatment (at the start of treatment)**

**Suspected gram negative infection** ☐

**Proven (microbiologically documented) gram negative infection*** ☐

**Proven multi-resistant gram negative infection** ☐

**With proven Meningitis** ☐

**With suspected Meningitis** ☐

**Renal sparing** ☐

***Blood culture**

**Summary of ciprofloxacin administration (since birth to 42 days after the study start)**

**Study period**

**Start of treatment** |__|__|/|__|__|/|__|__|__|__| (dd/mm/yyyy)

**End of treatment** |__|__|/|__|__|/|__|__|__|__| (dd/mm/yyyy)

| Everyday from the start to the end of treatment | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Dose number** | **Not administered*** | **dose mg** | **Date (day/ month/ year)** | **Start time of infusion** | **Stop time of infusion **** | **Line primed with ciprofloxacin Yes or no** | **State if given via a broviac** |
| **1** | | | | | | | |
| **2** | | | | | | | |
| **3** | | | | | | | |
| **4** | | | | | | | |
| **5** | | | | | | | |
| **6** | | | | | | | |
| **7** | | | | | | | |
| **8** | | | | | | | |
| **9** | | | | | | | |
| **10** | | | | | | | |
| **11** | | | | | | | |
| **12** | | | | | | | |
| **13** | | | | | | | |
| **14** | | | | | | | |
| **15** | | | | | | | |
| **16** | | | | | | | |
| **17** | | | | | | | |
| **18** | | | | | | | |
| **19** | | | | | | | |
| **20** | | | | | | | |
| **21** | | | | | | | |

*If the ciprofloxacin was not administrated code reason a) no intravenous access b) absent due to other procedure (theatre etc), **c) other describe (includes stopped due to end of treatment)**

****if the stop time has not been completed then check the nursing notes for any record of a delay in the infusion then make a note in the comments to say there is /is not a record of a delay and the planned infusion time (30 or 60 minutes) .**

**Summary of all treatment episodes from birth to 42 days after study start**

**Episode 1**

**Ciprofloxacin administered from ……to……Dose administered…….**

**Episode 2**

**Ciprofloxacin administered from ……to……Dose administered……..**

**Episode 3**

**Ciprofloxacin administered from ……to……Dose administered……..**

**Comments:**

**Important if administered via a Broviac the time the drug reaches the vein may need adjusting please state here the date and time doses were given via a broviac**

**Co-administration**

Record all antimicrobials, steroids, vasoactive, proton pump inhibitors, antiepileptics, inotrope, cox inhibitors (non-steroidal), albumin , Selective decontamination of the digestive tract and caffeine from the first dose of study drug and through the last dose of study drug therapy.

| Class | Drug name | Date of inclusion or start date if after inclusion | Stop date up to the end of study drug or state 'continuing' |
|---|---|---|---|
| **Antimicrobials** | | | |
| | | | |
| **Steroids** | | | |
| | | | |
| **Inotropic drugs** | | | |
| | | | |
| **Proton pump inhibitor ( antacids)** | | | |
| | | | |
| **Antiepileptics** | | | |
| | | | |
| **Warfarin** | | | |
| **Cox inhibitors (non-steroidal)** | | | |
| | | | |
| **Albumin** | | | |
| | | | |
| **Selective decontamination of the** | | | |
| **Indomethacin** | | | |
| **Diuretics** | | | |

☐ Caffeine

| Date of inclusion | Stop Date or last day of study drug | Mode of administration | Loading Dose | Maintenance dose | Interval between doses |
|---|---|---|---|---|---|
| | | | | | |

**PK Blood Sample Planner and Record of Collection Times**

1st Day of PK : |__|__|/|__|__|/|__|__|__|__| (dd/mm/yyyy)

Last day of PK |__|__|/|__|__|/|__|__|__|__| (dd/mm/yyyy)

Weight start of ciprofloxacin.....tinn
Weight Last day of PK sampling .....
Day of ciprofloxacin treatment: day 1☐ or day 2,☐, then day 5☐, day 6☐ or day 7☐,
Most recent weight (start of ciprofloxacin) : weight drug calculated on:
**Weight (day of PK (if available)**
Sample group (allocation): ☐ A ☐ B ☐ C ☐ D

| | | | | |
|---|---|---|---|---|
| **Q 12 h** | **A** | **T1** | **T3h** | **T8h** |
| | **B** | **T2h** | **T6h** | **T12h** |
| **Q8 h** | **C** | **T1h** | **T3h** | **T6h** |
| | **D** | **T2h** | **T4h** | **T8h** |

For weight< 1000g, 2 samples will be randomly selected from 3 predefined samples times (web site)

| | | | | |
|---|---|---|---|---|
| **Q 12 h** | **Ai** | **T1h** | **T3h** | |
| | **Aii** | **T1h** | | **T8h** |
| | **Aiii** | | **T3h** | **T8h** |
| | **BI** | **T2h** | **T6h** | |
| | **Bii** | **T2h** | | **T12h** |
| | **Biii** | | **T6h** | **T12h** |

## PK Blood Samples

| Planned Sample Schedule (according to allocated group) | Planned Sample Time | Actual Time sample collected | Date and time sample frozen at -20°C *(must be within 24 hours of collection)** | Sample source capillary /central venous/ arterial | Sample number** |
|---|---|---|---|---|---|
| **PK Day 1 or 2** | | | | | |
| **T….** | | | | | |
| **T….** | | | | | |
| **T….** | | | | | |
| **PK Last day** | | | | | |
| **T….** | | | | | |
| **T….** | | | | | |
| **T….** | | | | | |

**Christine Chesters Biochemistry will provide this data**
**** this does not allow letters so just put 1, 2 , 3 etc**
**T1 sample is required within 3 minutes of the end of the 1st infusion; other sample times allow 10 minutes before or after the set time.**

**PK Scavenged Samples - Blood samples (centrifugation before storage)**

| Date | Time collected | Date and Time frozen | sample number |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

**CSF Sample**

| Date | Time collected | Time frozen | sample number |
|---|---|---|---|
| | | | |
| | | | |

**DNA sample (buccal smears)**

| Date collected | Date completed *Extracted | sample number |
|---|---|---|
| | | |

**DNA sample (blood sample)**

**Received in batches from the lab so check lab have collected surplus FBC and state this date**

| Dates collected | Date completed * extracted | sample number |
|---|---|---|
| | | |

**Microbiological data - Culture Results**

**Data reported 72 hours prior to start of ciprofloxacin until day 10**

**Include most recent gram negative blood culture if earlier than this period. MIC reported at the end of the study**

| Site of collection using code list | Date | Organism (state if no growth) | Sensitivity /Resistance to ciprofloxacin | MIC Ciprofloxacin |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

**Code list :**

**1 Peripheral (blood) 2 Central line (blood) 3 Peritoneal fluid 4 Endotracheal tube suction 5 Other sterile (specify)**

**Faeces**

| Sample date | Date obtained /received: | Results:* | MIC |
|---|---|---|---|
| 1st Sample (immediately prior to ciprofloxacin or before day 2) | | Colonisation: | |
| 2nd Sample<br><br>Date packaging sent to family<br><br>…….<br>Required 4-6 weeks following ciprofloxacin | | Gram negative insensitivity: | |

**Results will be added at the end of the study so leave blank and do not freeze this form yet**

**Articular Phlebitis Assessment**

| Date | Examination | Assessment (indicate specially if it's related with ciprofloxacin)* Or enter not applicable |
|---|---|---|
| Prior to ciprofloxacin start | Normal or no clinical sign ↑<br>Pain ↑<br>Redness ↑<br>Immobility | |
| Day 5 | Normal or no clinical sign ↑<br>Pain ↑<br>Redness ↑<br>Immobility | |
| Day 7 | Normal or no clinical sign ↑<br>Pain ↑<br>Redness ↑<br>Immobility | |
| End of ciprofloxacin | Normal or no clinical sign ↑<br>Pain ↑<br>Redness ↑<br>Immobility ↑ | |
| End of hospitalisation | Normal or no clinical sign ↑<br>Pain ↑<br>Redness ↑<br>Immobility | |
| Any additional day if abnormal | Normal or no clinical sign ↑<br>Pain ↑<br>Redness ↑<br>Immobility | |

**Assessment of Phlebitis - Peripheral Infusion Site - VIP Score every day until the end of ciprofloxacin treatment – record the highest score for the vein that day.**

| Day of ciprofloxacin treatment Date | Planned Duration of infusion (30 minutes or 60 minutes) | Pre dose | Mid infusion | 1 hour post dose | 6 hours post dose | Central line infusion* |
|---|---|---|---|---|---|---|
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |

***IMPORTANT - If given via a broviac please make a comment in the summary as the drug may take longer to enter the vein.**

## Lab results

**If more than one blood sample has been taken in the day record the value that is furthest from the normal range Do not record decimal places for haemoglobin or CRP.**

| | **72 hours prior to ciprofloxacin Date:** | **1st day of ciprofloxacin Date:** | **Last study day of ciprofloxacin (day 5,6 or 7) Date:** | **3 days after last dose Date** |
|---|---|---|---|---|
| **Full Blood Count** | | | | |
| Haemoglobin | | | | |
| WBC | | | | |
| Platelets | | | | |
| Haematocrit | | | | |
| **Biochemistry** | | | | |
| Creatinine | | | | |
| Albumin | | | | |
| **Blood Gas** | | | | |
| pH | | | | |
| PCO2 | | | | |
| PO2 | | | | |
| Glucose | | | | |
| Ionized Calcium | | | | |
| Lactate | | | | |

**Liver function and CRP need to be recorded only on the days assessed for clinical care**

| | **Within 72 hours prior to ciprofloxacin** | **1st day of ciprofloxacin** | **Day 2** | **Day 3** | **Day 4** | **Day 5** | **Day 6** | **Day 7** | **3 days after last dose** |
|---|---|---|---|---|---|---|---|---|---|
| **Liver Function** | | | | | | | | | |
| Total Bilirubin | | | | | | | | | |
| Conjugated Bilirubin | | | | | | | | | |
| AST | | | | | | | | | |
| ALT | | | | | | | | | |
| ALK Phosphate | | | | | | | | | |
| Amylase | | | | | | | | | |
| **CRP** | | | | | | | | | |

**Summary of clinical history**

**Date of hospitalization in the NICU …….**

**End of hospitalization ……..**

Status at admission: Inpatient/in born or transferred from another hospital

Weight: end of cipro treatment or on 10th day (if available):

Weight end of hospitalization:

**Status 6 weeks following commencement of ciprofloxacin date …… :**

Inpatient ☐ Transferred to another hospital ☐ **Discharged Home:** ☐ **Death date**……..

**Ventilation**

**1st day ventilation from birth ……. Last Day of ventilation *……**

| **Ventilation** | **Mode on Day 1 PK samples** | **Last Day PK samples** |
|---|---|---|
| **HFO** | | |
| **Pressure support /BIPAP** | | |
| **CPAP** | | |
| **Non invasive CPAP** | | |
| **Not ventilated** | | |

- **If ventilation has stopped and started as part of weaning record the last day ventilated**

**Record transfusions from the 1st day of ciprofloxacin until day 10**

**Include Blood Product:** Packed Red Cells Fresh Frozen Plasma Cryo Human Albumin Solution Platelets

| **Date administered** | **Blood Product** | **Volume (ml)** |
|---|---|---|
| | | |
| | | |
| | | |

| **PDA** | **Yes or No** | | |
|---|---|---|---|
| Surgery | | **Date:** | |
| Indomethacin | | **Start date:** | **End date:** |
| Ibuprofen | | | |
| Prostin | | | |

| Intervention | 1st Day PK Samples | Last Day PK Samples (day 5, 6, or 7) |
|---|---|---|
| **Nutrition**<br>**Parenteral**<br>**Breast Milk**<br>**Formula** | <br>☐<br>☐<br>☐ | <br>☐<br>☐<br>☐ |
| **Gastroesophageal reflux** | ☐ | ☐ |
| **Phototherapy** | ☐ | ☐ |
| **Haemofiltration *** | ☐ | ☐ |
| **ECMO*** | ☐ | ☐ |
| **Cooling** | ☐ | ☐ |

***Patients on ECMO or Haemofiltration are excluded from the study but this is recorded if started after inclusion**

**Outcome record from 1st dose until 42 days (if the outcome occurs out with this time period then please add details to the comments box)**

| Outcome | Yes or No | Details | Date (within monitoring period) |
|---|---|---|---|
| **Surgery –any surgical procedure** | | | |
| **Necrotising Enterocolitis** | | **Surgical** ☐<br>**Medical** ☐ | |
| **Tolerability:–**<br>**state if ciprofloxacin was stopped due to a clinical concern** | | **Reason stopped:** | |
| **Fitting** | | | |
| **Intra ventricular Haemorrhage** | | **Grade I** ☐<br>**II** ☐<br>**III** ☐<br>**IV** ☐<br>**Grade not recorded** ☐ | |

**Comments**

*Include if any information:*
*To explain a deviation from the protocol*
*Chronic clinical condition that may affect drug metabolism such as Downs, Cystic fibrosis, hydrops*
**Include significant adverse events such as NEC, Death etc that occurred after the 42 day reporting period**

**Section 4**

**Pharmacovigilance Reporting commences on the 1st day of ciprofloxacin until 42 days afterwards (last study procedure) see Protocol Section 7 and SOP Section 7**

**Serious Adverse Events Reactions or Suspected Serious Adverse Events or Reactions require expedited reporting except for the Expected Serious Adverse Events listed in the protocol. These are classed as:**

Result in death (excluded from expedited reporting in this patient group*)

Life-threatening **

Requires hospitalisation or prolongation of existing hospitalization

Results in persistent or significant disability or incapacity

*(the subject was at risk of death at the time of the event it does not refer to an event which hypothetically might have caused death if it were more severe).

***Expected events that are relevant in this context will be collected systematically in the CRF (Death NEC, PDA) but do not require further reporting.**

****Adverse events that have been identified as more likely in the risk assessment of the drug will be collected systematically in the case report form. Additional pharmacovigilance reporting will only be required if the investigator assesses the event as serious including:**

**Arthropathy /Phlebitis / Liver Function /pancreatitis/Renal Function/Deranged Blood cells**

**Adverse events have been identified in the risk assessment as uncommon and should also be included:**

Anaphylaxis /Allergy

Skin: Rash/photosensitivity

Cardiac Arrhythmias: ventricular arrhythmia, QT interval prolongation

Syndromes: Stevens Johnson /Lyell

Neurological: convulsions

**Adverse event categorie:**
**Adverse event details**
**Start date:**
**Stop date:**
**Action taken with study drug:**
**Outcome:**
**Intensity:**
**Severity:**

**General comments**

## Adverse Events

Did subject have any non-serious adverse events related to study drug from first dose through 72 hours after last dose or any serious adverse events from first dose through 30 days after last dose? ☐ 0 No ☐ 1 Yes → If Yes: Complete below.

| # | Adverse Event | Start Date | Stop Date OR ☑ if Continuing | Relationship to Study Drug | Action Taken with Study Drug | Outcome | Intensity | Was This Event Serious? |
|---|---|---|---|---|---|---|---|---|
| 1 | | __ __/__ __ __/__ __ __ __ day month year | __ __/__ __ __/__ __ __ __ day month year OR ☐ 1 Continuing | ☐ 1 Definitely not related ☐ 2 Probably not related ☐ 3 Probably related ☐ 4 Definitely related | ☐ 1 None ☐ 2 Discontinued drug temporarily ☐ 3 Discontinued drug permanently | ☐ 1 Resolved ☐ 2 Resolved with sequelae ☐ 3 Not resolved ☐ 4 Death ☐ 5 Unknown | ☐ 1 Mild ☐ 2 Moderate ☐ 3 Severe ☐ 4 Life threatening | ☐ 0 No ☐ 1 Yes |
| 2 | | __ __/__ __ __/__ __ __ __ day month year | __ __/__ __ __/__ __ __ __ day month year OR ☐ 1 Continuing | ☐ 1 Definitely not related ☐ 2 Probably not related ☐ 3 Probably related ☐ 4 Definitely related | ☐ 1 None ☐ 2 Discontinued drug temporarily ☐ 3 Discontinued drug permanently | ☐ 1 Resolved ☐ 2 Resolved with sequelae ☐ 3 Not resolved ☐ 4 Death ☐ 5 Unknown | ☐ 1 Mild ☐ 2 Moderate ☐ 3 Severe ☐ 4 Life threatening | ☐ 0 No ☐ 1 Yes |
| 3 | | __ __/__ __ __/__ __ __ __ day month year | __ __/__ __ __/__ __ __ __ day month year OR ☐ 1 Continuing | ☐ 1 Definitely not related ☐ 2 Probably not related ☐ 3 Probably related ☐ 4 Definitely related | ☐ 1 None ☐ 2 Discontinued drug temporarily ☐ 3 Discontinued drug permanently | ☐ 1 Resolved ☐ 2 Resolved with sequelae ☐ 3 Not resolved ☐ 4 Death ☐ 5 Unknown | ☐ 1 Mild ☐ 2 Moderate ☐ 3 Severe ☐ 4 Life threatening | ☐ 0 No ☐ 1 Yes |
| 4 | | __ __/__ __ __/__ __ __ __ day month year | __ __/__ __ __/__ __ __ __ day month year OR ☐ 1 Continuing | ☐ 1 Definitely not related ☐ 2 Probably not related ☐ 3 Probably related ☐ 4 Definitely related | ☐ 1 None ☐ 2 Discontinued drug temporarily ☐ 3 Discontinued drug permanently | ☐ 1 Resolved ☐ 2 Resolved with sequelae ☐ 3 Not resolved ☐ 4 Death ☐ 5 Unknown | ☐ 1 Mild ☐ 2 Moderate ☐ 3 Severe ☐ 4 Life threatening | ☐ 0 No ☐ 1 Yes |
| 5 | | __ __/__ __ __/__ __ __ __ day month year | __ __/__ __ __/__ __ __ __ day month year OR ☐ 1 Continuing | ☐ 1 Definitely not related ☐ 2 Probably not related ☐ 3 Probably related ☐ 4 Definitely related | ☐ 1 None ☐ 2 Discontinued drug temporarily ☐ 3 Discontinued drug permanently | ☐ 1 Resolved ☐ 2 Resolved with sequelae ☐ 3 Not resolved ☐ 4 Death ☐ 5 Unknown | ☐ 1 Mild ☐ 2 Moderate ☐ 3 Severe ☐ 4 Life threatening | ☐ 0 No ☐ 1 Yes |

**Consent Withdrawn by Parent**

Date of Withdrawn : |__|__|/|__|__|/|__|__|__|__| (dd/mm/yyyy)

**Name of Parent:** **Date consent withdrawn:**

Describe whether consent is withdrawn from the PK safety /sampling study, from CSF, DNA or all. Confirm with parent if the data already collected may still be used.

**Name of Person that discussed withdrawal of consent with the parent ……..**

**----------**

**Case report From Completed by:**

**Name:** **Signature:** **Date:**

# APPENDIX III Results – Pharmacokinetic Parameters

| **PMA** | | **CL** (L/h) | **V1** (L) | **V2** (L) | **Q** (L/h) | **AUC** $_{0\text{-}24}$ (mg*h/L) | **T1/2 beta** (h) | **Dose** (mg/ dose) | **Dose** (mg /kg /dose) | **PMA** week | **PNA** (day) | Current weight (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 24-27 | N | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| | Mean | 0.10 | 0.82 | 0.85 | 1.32 | 179 | 15.64 | 7.3 | 8.7 | 27 | 14 | 831 |
| | Median | 0.08 | 0.84 | 0.85 | 1.34 | 167 | 12.60 | 7.7 | 9.6 | 27 | 12 | 850 |
| | Min | 0.04 | 0.54 | 0.64 | 1.16 | 85 | 5.70 | 4.5 | 5.0 | 25 | 3 | 700 |
| | Max | 0.22 | 1.08 | 1.15 | 1.42 | 289 | 33.80 | 9.2 | 10.0 | 28 | 27 | 915 |
| | Std. | 0.06 | 0.18 | 0.18 | 0.10 | 78 | 9.07 | 1.7 | 1.7 | 1 | 8 | 84 |
| 28-31 | N | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | Mean | 0.17 | 1.24 | 1.33 | 1.69 | 130 | 13.06 | 10.0 | 8.7 | 30 | 24 | 1154 |
| | Median | 0.15 | 1.18 | 1.24 | 1.64 | 115 | 12.65 | 9.3 | 9.5 | 30 | 24 | 1090 |
| | Min | 0.11 | 0.58 | 0.73 | 1.34 | 65 | 5.60 | 5.4 | 4.4 | 28 | 6 | 850 |
| | Max | 0.30 | 2.0 | 3.12 | 2.31 | 291 | 23.20 | 18.0 | 10.6 | 31 | 43 | 1755 |
| | Std. | 0.06 | 0.44 | 0.66 | 0.27 | 60 | 5.85 | 3.4 | 2.0 | 1 | 9 | 243 |
| 32-35 | N | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| | Mean | 0.24 | 1.40 | 1.44 | 2.04 | 132 | 12.09 | 12.5 | 8.3 | 33 | 31 | 1492 |
| | Median | 0.18 | 1.44 | 1.48 | 1.95 | 136 | 9.10 | 12.7 | 9.6 | 33 | 30 | 1400 |
| | Min | 0.08 | 0.30 | 0.26 | 1.66 | 73 | 2.80 | 5.7 | 5.0 | 31 | 3 | 1130 |
| | Max | 0.46 | 3.22 | 2.82 | 2.63 | 207 | 32.10 | 20.0 | 10.1 | 35 | 56 | 2090 |
| | Std. | 0.16 | 0.89 | 0.70 | 0.30 | 43 | 9.77 | 4.6 | 2.3 | 1 | 19 | 300 |
| 36-39 | N | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 |
| | Mean | 0.54 | 2.61 | 2.54 | 2.88 | 114 | 7.91 | 22.0 | 9.4 | 38 | 46 | 2362 |
| | Median | 0.48 | 2.25 | 2.39 | 2.92 | 113 | 7.10 | 23.0 | 10.0 | 37 | 45 | 2400 |
| | Min | 0.26 | 1.75 | 1.57 | 2.34 | 46 | 4.10 | 18.0 | 6.3 | 36 | 4 | 1790 |
| | Max | 1.04 | 4.44 | 5.02 | 3.61 | 178 | 14.00 | 27.0 | 11.0 | 41 | 105 | 3190 |
| | Std. | 0.24 | 0.82 | 0.86 | 0.38 | 39 | 3.14 | 3.2 | 1.2 | 1 | 35 | 420 |
| 40-43 | N | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | Mean | 1.01 | 3.04 | 2.76 | 3.44 | 95 | 4.89 | 27.3 | 9.3 | 42 | 52 | 2962 |
| | Median | 1.09 | 3.13 | 2.74 | 3.53 | 64 | 4.90 | 28.5 | 9.9 | 42 | 30 | 3053 |
| | Min | 0.44 | 1.15 | 1.68 | 2.64 | 41 | 2.70 | 15.0 | 5.8 | 41 | 9 | 2100 |
| | Max | 1.45 | 4.38 | 4.35 | 4.16 | 201 | 9.00 | 35.0 | 11.0 | 44 | 122 | 3850 |
| | Std. | 0.39 | 0.95 | 0.75 | 0.49 | 51 | 2.00 | 6.3 | 1.7 | 1 | 42 | 545 |
| 44-48 | N | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| | Mean | 1.36 | 3.85 | 3.42 | 3.81 | 88 | 4.66 | 32.3 | 9.3 | 46 | 58 | 3448 |
| | Median | 1.20 | 4.25 | 3.34 | 3.99 | 73 | 5.10 | 31.5 | 9.4 | 46 | 52 | 3650 |
| | Min | 0.49 | 2.21 | 2.16 | 2.81 | 35 | 2.10 | 20.0 | 8.3 | 43 | 31 | 2280 |
| | Max | 3.42 | 5.44 | 5.51 | 4.44 | 184 | 6.60 | 40.0 | 10.0 | 48 | 108 | 4200 |
| | Std. | 0.88 | 1.28 | 1.11 | 0.60 | 44 | 1.46 | 7.5 | 0.6 | 2 | 28 | 712 |
| Total | N | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| | Mean | 0.56 | 2.17 | 2.08 | 2.54 | 122 | | 18.9 | 9.1 | 35.7 | 38 | |
| | Median | 0.38 | 1.92 | 1.99 | 2.51 | 110 | | 18.7 | 9.7 | 36.5 | 27 | |
| | Min | 0.04 | 0.30 | 0.26 | 1.16 | 35 | 2.10 | 4.5 | 4.4 | 24.9 | 5 | 700 |
| | Max | 3.42 | 5.44 | 5.51 | 4.44 | 291 | 33.8 | 40 | 11 | 47.8 | 121 | 4200 |
| | Std. | 0.58 | 1.31 | 1.14 | 0.96 | 60 | | 10.1 | 1.6 | 6.5 | 30 | |

**APPENDIX IV**

# Appendix IV Clinical Outcome Protocol

## The minimum inhibitory concentration of Ciprofloxacin and its clinical utility in neonates with Gram Negative septicaemia MT 201108

MA Turner, S Thiesen, S Foulkes, A Hart, C Parry, T Neal

### Background

Ciprofloxacin is a fluoroquinolone antibiotic with useful activity against Gram Negative bacteria [Andriole 2005].

It has been used as a second line antibiotic on the Neonatal Unit at Liverpool Women's Hospital for 20 years [Bannon 1989]. This Neonatal Unit admits over 1000 newborn babies a year of whom 60 – 70 are given ciprofloxacin. Organisms isolated from neonatal blood cultures are retained in the Clinical Microbiology laboratories at Royal Liverpool University Hospital. Most ciprofloxacin is administered because of clinical suspicion of sepsis but there have been about 150 cases of proven Gram Negative sepsis during the past 15 years. Most of these cases have been given ciprofloxacin. The use of ciprofloxacin on this large neonatal unit has avoided problems relating to cephalosporin resistance that have been experienced by other units.

Ciprofloxacin is off-patent and is used off-label in neonates. As such the European Medicines Agency (EMEA) identified it as a priority medicine for work to develop a "Paediatric Marketing Use Authorisation" (PUMA). A consortium involving Liverpool Women's Hospital has been funded by the European Commission to prepare an application for a PUMA. Dr. Turner is leading the development and implementation of a paediatric investigation plan (PIP) for ciprofloxacin. As part of the work towards the PIP and PUMA we wish to examine our use of ciprofloxacin in some detail.

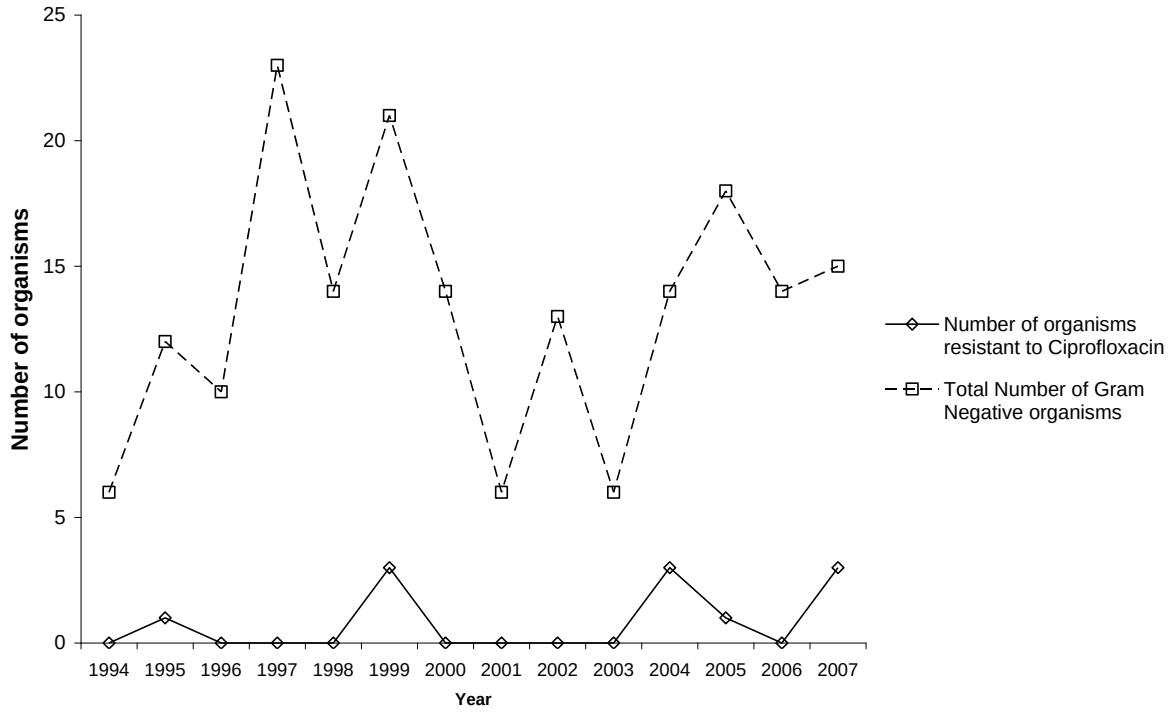
An important aspect of the evaluation of any antibiotic is the extent of resistance to the antibiotic among the target organisms. Resistance can be quantified as the "Minimum Inhibitory Concentration" (MIC) which is measured under standardised conditions and indicates how much antibiotic is required to inhibit visible growth of a

micro-organism. A low MIC indicates a susceptible, or sensitive, micro-organism. A high MIC indicates that a micro-organism is resistant to a particular antibiotic. Recent work on the mechanisms of resistance to ciprofloxacin has identified several types of resistance [Robicsek 2006]. Some of these are associated with a clear signal of resistance on routine laboratory testing, with a high MIC. We have examined resistance to ciprofloxacin for isolates obtained from blood culture from neonates in Liverpool. The results are shown in Table 1. We found that the extent of resistance appears to differ according to year of isolate (Figure 1).

*Table 1: sensitivity to ciprofloxacin of Gram Negative organisms isolated from blood cultures in neonates in Liverpool 1994 – 2007.*

| **Organism** | **Total** | **Sensitive (N)** | ***Sensitive (%)*** | **Resistant (N)** | ***Resistant (%)*** |
|---|---|---|---|---|---|
| Acineobacter baumannii | 2 | 1 | *50* | 1 | *50* |
| Acinetobacter | 1 | 1 | *100* | 0 | *0* |
| Acineobacter lwoffii | 5 | 4 | *80* | 1 | *20* |
| Enterobacter amnigenus | 2 | 2 | *100* | 0 | *0* |
| Enterobacter cloacae | 19 | 19 | *100* | 0 | *0* |
| Enterobacter spp | 6 | 6 | *100* | 0 | *0* |
| E coli | 56 | 54 | *96* | 2 | *4* |
| Klebsiella oxytoca | 10 | 10 | *100* | 0 | *0* |
| Klebsiella pneumoniae | 10 | 8 | *80* | 2 | *20* |
| Proteus mirabilis | 3 | 3 | *100* | 0 | *0* |
| Pseudomonas aeruginosa | 31 | 29 | *94* | 2 | *6* |
| Pseudomonas versicularis | 1 | 0 | *0* | 1 | *100* |
| Serratia marcescens | 11 | 11 | *100* | 0 | *0* |
| Stenotrophomonas maltophilia | 2 | 1 | *50* | 1 | *50* |
| **Total** | **159** | **149** | ***94*** | **10** | ***6*** |

*Figure 1. Number of Gram Negative organisms resistant to ciprofloxacin in Liverpool Neonatal Units by time.*




Some types of resistance are associated with more subtle changes in the susceptibility to ciprofloxacin and these are associated with smaller changes in MIC [Robicsek 2006]. This low level resistance may be more easily detected by testing the isolate against the lower class quinolone agent; nalidixic acid. In other populations it has been suggested that subtle changes in MIC may predispose to resistance and possibly to a reduced effectiveness of fluoroquinolones [Chau 2007]. However, this issue has not yet been examined in neonates.

The value of an antibiotic can be examined in two ways. The “effectiveness” describes whether it is better than a comparator (another antibiotic or placebo). The “utility” describes what happens when an antibiotic is used in individual patients. The ethical and scientific complexities inherent to randomising an infant with a life-threatening infection that may be due to a multiply-resistant micro-organism mean that it is not

feasible to examine the effectiveness of ciprofloxacin for this patient population. Accordingly, we are concerned here with the utility of ciprofloxacin in neonates.

The overall objective of this study is to examine how subtle changes in MIC are related to the utility of ciprofloxacin in neonates with Gram Negative septicaemia. We will also examine the clinical course of infants who have had a Gram Negative septicaemia.

The storage of isolates is incomplete, particularly for the earlier years of this series. Sixty five isolates are available. This means that a limited number of infants will contribute to studies of the relationship between MIC and clinical outcome. Nevertheless, descriptive data for infants with Gram Negative isolates on blood cultures will inform the design of further studies. Thus, for infants whose isolates have not been retained, we will examine the clinical data. For these reasons we will consider the cohort in two ways: a) those who have complete data for both microbiological and clinical aspects and are eligible for consideration in the primary hypothesis (these are identified by a “Case Definition”); b) all infants with clinical data available (these are identified by “Inclusion criteria”). This is in accord with guidance from the European Medicines Agency about the evaluation of antibiotics [EMEA, 2004].

**Aims**

1. To examine the relationship between MIC of ciprofloxacin with respect to Gram Negative organisms and the rate of clinical cure of Gram Negative septicaemia.

2. To determine whether MIC of ciprofloxacin is related to other indicators of utility including the number of days during which the infant had evidence of inflammation, the duration of ciprofloxacin therapy, microbiological cure rates and the use of other antibiotics.

3. To describe the clinical course of Gram Negative septicaemia in all available cases.

**Hypotheses**

1. Among neonates who receive ciprofloxacin to treat a Gram Negative organism a higher MIC to nalidixic acid is associated with less likelihood of clinical cure.

2. Among neonates who receive ciprofloxacin to treat a Gram Negative organism a higher MIC to nalidixic acid is associated with longer duration of an inflammatory state, longer administration of ciprofloxacin, lower microbiological cure rates and increased use of other antibiotics.

The third aim will yield descriptive data and a hypothesis will not be tested.

**Outcomes and other variables to be assessed**

*Primary exposure variable:*

MIC of ciprofloxacin to Gram Negative organism

*Primary outcome variable:*

Clinical cure: survival to the end of an episode of sepsis during which a Gram Negative bacterium was isolated where the episode of sepsis ended within 10 days of starting ciprofloxacin

The end of the episode of sepsis during which the Gram Negative organism was isolated will be defined as the day on which the C-reactive protein (CRP) is first < 10mg/l. CRP is measured daily on sick neonates on the neonatal unit. The cut-off of 10 days has been selected on the basis of clinical experience. The time to recovery for each infant will be assessed as a secondary outcome.

*Important covariates*

These have been selected to capture factors that are associated with poor clinical outcomes and which could thus mask the effect of ciprofloxacin on the primary outcome. Not all of these covariates will be included in the final model, but in this exploratory study we need to collect data in order to find the best summary of the situation.

Gestational age at birth. Justification: infants born at lower gestational ages are more likely to die from sepsis and more likely to have co-morbidities.

Sex. Justification: male infants generally do worse than females.

Nature of illness at start of ciprofloxacin treatment (septicaemia, meningitis, NEC, other). Justification: infants with co-morbidity are more likely to require longer courses of ciprofloxacin.

Illness severity at start of ciprofloxacin treatment (assessed using Neonatal Therapeutic Intervention Scoring System – NTISS – see Appendix 1). Justification: infants with co-morbidity are more likely to require longer courses of ciprofloxacin.

Year of blood culture

*Secondary outcomes:*

Demographics (including assessment of relationship between year of isolate and MIC) will be done on all eligible isolates and descriptive statistics relating to the clinical course of episodes of Gram Negative sepsis will be prepared.

For each infant:

Number of days of the inflammatory state (CRP > 9.9 mg/L; platelet count < 100 x $10^{12}$), i.e. time to recovery.

Number of days of ciprofloxacin treatment

Prescription of other antibiotics targeted at Gram Negative bacteria

Persistence of the same organism in blood cultures taken more than 72 hours after start of ciprofloxacin treatment

Recurrence of the same organism in blood cultures after the end of treatment

Blood culture positive to another Gram Negative organism.

Survival to discharge

Condition at discharge: presence of Grade 3/4 IVH or periventricular leucomalacia; oxygen requirement at 36 weeks corrected gestational age.

**Inclusion criteria for data collection**

Infants who were in-patients in a Liverpool Neonatal Unit between 1994 and 2008 who were reported to have Gram Negative isolates by the Microbiology laboratories at Royal Liverpool University Hospital.

**Case definition for inclusion in models analysis relating to the primary hypothesis**

Blood culture positive for a Gram Negative organsism.

**Methods**

1. Laboratory records will be used to prepare a list of Gram Negative isolates obtained from blood cultures performed on infants who were in-patients at a Liverpool Neonatal Unit and who have isolates stored in the microbiology laboratory
2. The relevant infant will be identified. Each infant will only contribute once to the analysis of the primary hypothesis, using the first episode of Gram Negative sepsis if there is more than one episode.
3. Descriptive data about subsequent episodes of sepsis will also be gathered and summarised.
4. MIC to ciprofloxacin and susceptibility to nalidixic acid will be measured using standard methods by Dr Foulkes.

5. Clinical notes will be obtained and data extracted using a standardised proforma by Dr. Thiessen.
6. A dataset with no identifying data will be prepared for analysis. Each patient will be assigned a unique study number and a code-break sheet will be retained (linked anonymised data).
7. Cases that are evaluable for the primary hypothesis will be idendified.
8. Data analysis will be performed by Dr. Thiessen and Dr. Turner with input from Mrs. Hart.

**Sample size**

This is a sample of convenience.

*Whole sample*

Up to 150 cases of Gram Negative septicaemia are available.

*Evaluable cases*

We anticipate that 65 isolates will be eligible. Of these 30 are likely to have died, have an episode of sepsis that lasts more than 10 days or be prescribed another antibiotic targeted at Gram Negative bacteria. This gives an estimate of 30 neonates who will have a clinical cure.

a) For a logistic regression model the dataset is likely to be sufficient to construct a model with 3 covariates
b) Using standard assumptions about regression models this dataset is likely to be sufficient to construct a model with 5 covariates.

**Analytic plan**

*Characterisation of the whole sample*

Summary statistics of demographic variables and course of Gram Negative infections. Course of Gram negative infections will be assessed using a) C-reactive protein; b) platelet count; c) NTISS; d) a selection of markers from NTISS: mechanical ventilation, inotrope therapy,

Univariate correlations between MIC and year of isolate, gestational age at birth, number of days CRP > 9.9mg/l.

*Primary analyses*

Aim 1.

Dependent variable: death / administration of ciprofloxacin for more than 10 days

Possible independent variables: Year of isolate; MIC; Gestational age at birth; Sex; Presence of NEC; illness severity score at start of treatment.

Statistical approach: Logistic regression

Aim 2.

Dependent variable: number of days with CRP > 9.9 mg/l after starting ciprofloxacin.

Possible independent variables: Year of isolate; MIC; Gestational age at birth; Sex; Presence of NEC; illness severity score at start of treatment.

Regression analysis for all possible independent variables.

Survival analysis for continuous variables: MIC; Gestational age at birth; illness severity score at start of treatment.

For Aims 1 and 2 we will construct a range of models and make a clinically informed decision about which model best addresses our hypothesis about the relationship between MIC and the explanatory variables.

We will explore the relative importance of independent variables using bootstrapping to systematically examine which variables contribute to models of samples of the dataset.

Aim 3 (all cases).

Summary of clinical course for all available cases with point estimate and summary of variation for each primary outcome, covariate and secondary outcome.

These summaries will be tabulated according to subgroups:

Gestational age at birth: 23 – 25 weeks; 26 – 28 weeks; 29 – 32 weeks; 33 – 36 weeks; ≥ 37 weeks

*Secondary analyses*

These are designed to summarise the data in order to inform the design of future studies.

Aim 2  Descriptive data of the incidence of cure / failure rates according to different definitions of cure /failure

Sensitivity analysis of regression models using different variables.

Aim 3  Univariate analysis to explore whether covariates are associated with outcomes including:

Correlation between gestational age and each outcome.

**Ethical issues**

This analysis will not alter the care given to babies included in the study since they have either died or been discharged from the Unit.

This analysis will use data that was routinely collected in clinical practice. Identifiable data will be used to construct and clean the dataset but will not be used in the analysis.

Participants will have been born over a large time range (1994 – 2008) and many will have died.

Accordingly we believe that consent is not necessary, feasible or proportionate.

We will not seek individual consent.

**Resource issues**

Measurement of MIC will be done by Microbiology trainees using the time and resources set aside for research within their training programme.

Extraction of clinical data will be done by a Neonatal trainee using the time and resources set aside for research within her training programme.

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# Appendix V Publications and Presentations

## Publications

Zhao, W., Hill, H., Le Guellec, C., Neal, T., Mahoney, S., Paulus, S., Castellan, C., Kassai, B. van den Anker, J. N., Kearns, G. L., Turner, M. A., Jacqz-Aigrain, E.: Population pharmacokinetics of ciprofloxacin in neonates and young infants less than three months of age. Antimicrob Agents Chemother vol 58 no11 6572-80 2014

Sumatra Ray and Sue Fitzpatrick (H Hill author of Chapter)
Chapter Informed Consent in a Research Setting
Oxford Handbook of Clinical and Health Care Research 2016 publisher Oxford Medical Handbooks

Leroux S, Turner M, Le Guellec C, Hill H, van den Anker J, Kearnes G, Jacqz-Aigrain E, Zhao W, Pharmacokinetic Studies in Neonates: The Utility of an Opportunistic Sampling Design Clin Pharmacokinetics 2015

Hill H 2012
Risk Adaptive Approach Neonatal Pharmacokinetic Clinical Trials Model
Medicines and Health Care Products Agency (MHRA) web site http://forums.mhra.gov.uk/forumdisplay.php?22-Risk-adaptive-approach

## Oral Presentations

Hill H, Fisher A, and Francis G
European Medicine Agency International Conference
A risk based approach for clinical trials in neonates
MHRA London UK 2012

Hill H, Francis G and Fisher A
Medicines and Health Care Products Agency
Consultation - A risk based approach for clinical trials in Neonates
MHRA London UK 2012

H.Hill and Turner M.
Pharmacokinetic Clinical Trial of Ciprofloxacin
National Workshop on Paediatric Clinical Pharmacology, Nottingham University Derby UK 2012,

## Posters

Hill H and Turner M
Challenges of Neonatal Pharmacokinetic Clinical Trials
Paediatric Intensive Care Society London 2014

Hill H, Zhao W, Jaques-Aigrain E, Turner M
The Pharmacokinetics of Ciprofloxacin in Neonates
European Society for Paediatric Infectious Disease Milan 2013

Hill H, Hughes G, Jacques- Aigrain E J A, Abernethy L, Turner M
Intra subject variability on 3T MRI Imagery of Neonatal Hips
European Association of Paediatric Societies Istanbul Turkey 2012

Hill H, Turner M
Reducing Barriers to Clinical Trials – MHRA Risk Proportionate Approach
European Society of Paediatric Societies Istanbul Turkey 2012

Hill H, Kirkham J, Paulus S. Neal T, Turner M
Do we need paediatric breakpoints? Comparing the MIC and clinical outcome of Ciprofloxacin
Paediatric Intensive Care Society Dublin 2012

Hill H, Turner M
Reducing Barriers to Clinical Trials – A risk proportionate approach MHRA
Paediatric Intensive Care Society Dublin 2012

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# Population Pharmacokinetics of Ciprofloxacin in Neonates and Young Infants Less than Three Months of Age

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**Ciprofloxacin is used in neonates with suspected or documented Gram-negative serious infections. Currently, its use is off-label partly because of lack of pharmacokinetic studies. Within the FP7 EU project TINN (Treat Infection in NeoNates), our aim was to evaluate the population pharmacokinetics of ciprofloxacin in neonates and young infants <3 months of age and define the appropriate dose in order to optimize ciprofloxacin treatment in this vulnerable population. Blood samples were collected from neonates treated with ciprofloxacin and concentrations were quantified by high-pressure liquid chromatography–mass spectrometry. Population pharmacokinetic analysis was performed using NONMEM software. The data from 60 newborn infants (postmenstrual age [PMA] range, 24.9 to 47.9 weeks) were available for population pharmacokinetic analysis. A two-compartment model with first-order elimination showed the best fit with the data. A covariate analysis identified that gestational age, postnatal age, current weight, serum creatinine concentration, and use of inotropes had a significant impact on ciprofloxacin pharmacokinetics. Monte Carlo simulation demonstrated that 90% of hypothetical newborns with a PMA of <34 weeks treated with 7.5 mg/kg twice daily and 84% of newborns with a PMA ≥34 weeks and young infants receiving 12.5 mg/kg twice daily would reach the AUC/MIC target of 125, using the standard EUCAST MIC susceptibility breakpoint of 0.5 mg/liter. The associated risks of overdose for the proposed dosing regimen were <8%. The population pharmacokinetics of ciprofloxacin was evaluated in neonates and young infants <3 months old, and a dosing regimen was established based on simulation.**

Ciprofloxacin, a synthetic fluoroquinolone, can be used to treat sepsis caused by multiple resistant organisms (1). It is not considered a first-line treatment in current guidelines for neonatal sepsis but is used in severe infections caused by *Enterobacter* spp. resistant to standard treatment and when there is a major risk of meningitis and secondary cerebral abscess (2, 3). In a recent European survey, ciprofloxacin was used "off-label" in 25% of neonatal intensive care units, mainly in cases of culture-proven bacterial sepsis due to multidrug-resistant organisms that are sensitive to ciprofloxacin (4).

After intravenous administration, ciprofloxacin is widely distributed in most bodily fluids and tissues, with a high penetration in the cerebrospinal fluid (CSF) and central nervous system. Glomerular filtration and tubular secretion are the main mechanisms of renal excretion, and >65% of ciprofloxacin is excreted unchanged by the kidney (5). In adults, the area under the concentration-time curve from 0 to 24 h ($AUC_{0-24}$)/MIC ratio appears to be the best predictor of microbiological and clinical outcome. A target $AUC_{0-24}$/MIC value of 125 was required for treating Gram-negative infections (6, 7).

Since data in neonates are limited, the present study was conducted to assess the population pharmacokinetics of ciprofloxacin in neonates and young infants <3 months of age and to use these data to calculate an optimal dosing regimen of ciprofloxacin for use in these patients.

## MATERIALS AND METHODS

**Study design.** The trial was a prospective, open label pharmacokinetic study of ciprofloxacin, conducted in the neonatal intensive care unit of the Liverpool women's hospital and in the pediatric intensive care unit of the Alder Hey Children's Hospital, Liverpool, United Kingdom. Inclusion and exclusion criteria are presented in Fig. 1. The study was approved by the institutional ethics board and independent ethics board of the TINN project (EudraCT 2010-019955-23). It was also monitored by an independent safety monitoring board (DSMB).

**Dosing regimen and pharmacokinetic sampling.** Ciprofloxacin (generic; Peckforton Pharmaceuticals, Crewe, United Kingdom) was administered as an intravenous infusion either over either 30 or 60 min by using a syringe pump connected to microbore tubing at a dose of 10 mg/kg/dose twice daily (BID) for neonates with a postmenstrual age (PMA) of <40

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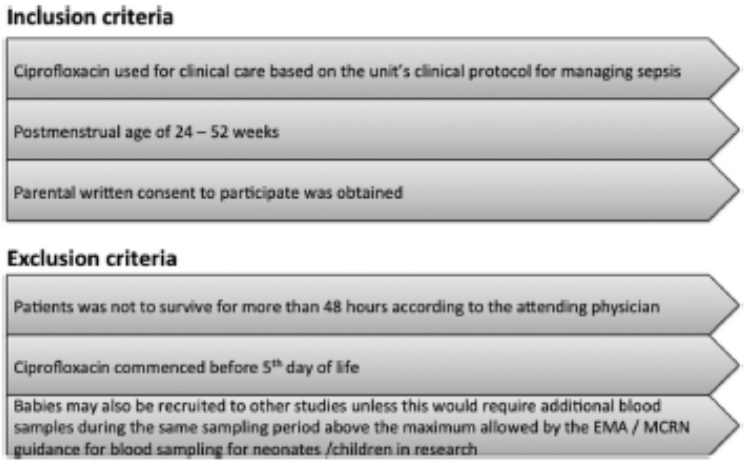


FIG 1 Inclusion and exclusion criteria.

weeks and three times daily (TID) or BID for young infants with a PMA of ≥40 weeks.

Ciprofloxacin pharmacokinetics was assessed on day 1 (D1) or D2 of treatment and again between D5 and D7. The total number of study-specific blood samples was restricted to six per participant with a maximum of three on each sampling day. Patients were randomly assigned to one of the two predefined three-time point schedules (Table 1). Precise infusion and sample times were recorded. The blood volume of samples obtained for pharmacokinetic analyses was 0.2 ml per sample. Scavenged samples were also obtained from blood remaining after routine biochemical tests. Only samples with validated sampling information were included. Blood samples were refrigerated and centrifuged (2,500 × *g* at 4C for 10 min), and serum or plasma was stored at −70°C. Samples were shipped on dry ice to the Department of Pediatric Pharmacology at Robert Debré Hospital, where they were stored at −70°C prior to analysis.

**Analytical method of ciprofloxacin and creatinine.** The analytical method of ciprofloxacin has been reported previously (8). Briefly, ciprofloxacin concentrations were determined using high-performance liquid chromatography with mass spectrometry with ciprofloxacin-$d_8$ as an internal standard. The calibration curve ranged from 25 to 3,000 ng/ml. The inter- and intraday coefficients of variation of controls were 4.1 and 2.4%, respectively. The lower limit of quantification was 25 ng/ml. Serum creatinine concentrations were measured by an adapted Jaffé method using the Architect C system (Abbott Diagnostics, Abbott Park, IL).

**Population pharmacokinetic modeling of ciprofloxacin.** Pharmacokinetic analysis was carried out using the nonlinear mixed effects modeling program NONMEM v7.2 (Icon Development Solutions, San Antonio, TX). First-order conditional estimation method with interaction was used to estimate pharmacokinetic parameters and their variability.

TABLE 1 Pharmacokinetic sampling schedule

| Group | Sampling time[a] | | | | | |
|---|---|---|---|---|---|---|
| Ciprofloxacin administered twice daily | | | | | | |
| A | End of infusion* | | T3 | | T8 | |
| B | | T2 | | T6 | | T12 |
| Ciprofloxacin administered three times daily | | | | | | |
| C | End of infusion* | | T3 | | | T8 |
| D | | T2 | | T4 | | T8 |

[a] The sampling times are indicated with reference to the start of ciprofloxacin infusion.
*, For infants weighing <1,000 g, samples were taken randomly on two specific times to minimize blood loss and to ensure representation of each period.

The interindividual variability of the pharmacokinetic parameters was estimated by using an exponential model and was expressed as follows: $\theta_i = \theta_{mean} * e^{\eta i}$, where $\theta_i$ represents the parameter value of the *i*th subject, $\theta_{mean}$ is the typical value of the parameter in the population, and $\eta i$ is the variability between subjects, which is assumed to follow a normal distribution with a mean of zero and variance ω2.

Covariate analysis followed a forward and backward selection process. The likelihood ratio test was used to test the effect of each variable on model parameters. The effects of current weight, birth weight, gestational age, postnatal age, postmenstrual age, serum creatinine concentration (collected within ≤48 h of pharmacokinetic sampling), and comedication were investigated as potential variables affecting pharmacokinetic parameters. During the first step of covariate model building, a covariate was included if a significant ($P < 0.05$, $\chi^2$ distribution with one degree of freedom) decrease (reduction > 3.84) in the objective function value (OFV) from the basic model and a reduction in the variability of the pharmacokinetic parameter were obtained. All of the significant covariates were then added simultaneously into a "full" model. Subsequently, each covariate was independently removed from the full model. If the increase in the OFV was higher than 6.635 ($P < 0.01$, $\chi^2$ distribution), the covariate was considered significantly correlated with the pharmacokinetic parameter and was therefore retained in the final model.

Model validation was based on graphical and statistical criteria. Goodness-of-fit plots, including observed (DV) versus population prediction (PRED), DV versus individual prediction (IPRED), conditional weighted residuals (CWRES) versus time, and CWRES versus PRED, were initially used for diagnostic purposes (9). The stability and performance of the final model was also assessed by means of a nonparametric bootstrap with resampling and replacement. Resampling was repeated 500 times, and the values of estimated parameters from the bootstrap procedure were compared to those estimated from the original data set. The entire procedure was performed in an automated fashion, using PsN (v2.30) (10). The final model was also evaluated graphically and statistically by normalized prediction distribution errors (NPDE) and prediction-corrected visual predictive check (pcVPC) (11, 12). One-thousand data sets were simulated using the final population model parameters. The NPDE results were summarized graphically by default as provided by the NPDE R package (v1.2) (13): (i) QQ-plot of the NPDE and (ii) histogram of the NPDE. The NPDE is expected to follow an "N(0,1)" distribution. For pcVPC, ob-

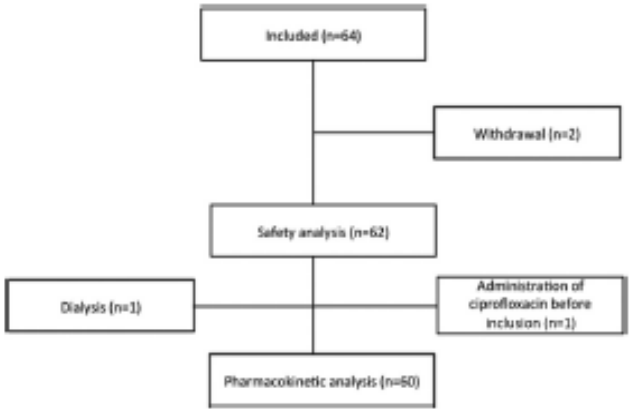


FIG 2 Trial flow chart.

served and simulated dependent variables were normalized based on the typical population prediction for the median independent variable in the bin. The 95% confidence intervals for the median and the 5th and 95th percentiles of the prediction-corrected simulated concentrations were calculated, plotted against the time, and compared to the prediction-corrected observed concentrations.

**Ciprofloxacin penetration into the CSF.** Assessment of the ciprofloxacin penetration into the CSF was evaluated by the CSF/serum ciprofloxacin concentration ratio. Because serum ciprofloxacin concentrations were not obtained concurrently with CSF sample collection, serum concentrations at the time of CSF sample collection were calculated via Bayesian estimation using the final model.

**Dosing regimen optimization.** Monte Carlo simulations were performed using the parameter estimates obtained from the final model in order to define optimal dosing regimen able to attain the target AUC/MIC value of 125 h in ca. 80% of patients. To ensure comparable safety profiles, the percentage of patients below the reported maximum AUC was also considered. The pediatric dose of ciprofloxacin was simulated on a mg/kg basis according to different age groups. Thus, various mg/kg dosing regimens (5, 7.5, 10, 12.5, and 15 mg/kg/dose BID) were simulated in each neonatal group. One-thousand simulations were performed using the original data set, and $AUC_{0-24}$ at steady state was calculated for each simulated patient. The target attainment rate was then calculated for each dosing regimen to define the optimal dose regimen in each neonatal group.

## RESULTS

**Study population.** Sixty-four patients were initially included from February 2011 to June 2012. All of the patients fulfilled the inclusion and exclusion criteria, and informed consent was obtained from all patients. Four patients were excluded from the pharmacokinetic analysis for the following reasons: two patients were withdrawn from the study, one patient was treated with dialysis, and one patient received ciprofloxacin within 36 h of inclusion. Finally, 60 newborns were included for the population pharmacokinetic analysis. Of these, 7 received ciprofloxacin at 5 mg/kg/dose BID, 6 were administered ciprofloxacin at 10 mg/kg/dose TID, and 47 were given ciprofloxacin at 10 mg/kg/dose BID. No patients discontinued the ciprofloxacin treatment due to adverse events, and no drug-related adverse events were shown to have a causal association with ciprofloxacin therapy. The trial flow is presented in Fig. 2.

The mean ± the standard deviation postmenstrual age (PMA) and weight of the 60 patients at the time of study were 35.7 ± 6.5 weeks (range, 24.9 to 47.9) and 2,060 ± 1,020 g (range, 700 to 4,200 g), respectively. The PMA and current weight were all normally distributed ($P = 0.4$ and $P = 0.2$, respectively [Kolmogorov-Smirnov test]). A summary of patient characteristics is presented in Table 2.

TABLE 2 Baseline characteristics in 60 neonates and infants

| Characteristics[a] | No. of patients | Mean (SD) | Median (range) |
|---|---|---|---|
| Total patients | 60 | | |
| Gender (male/female) | 39/21 | | |
| Race (Caucasian/Asian/unknown) | 53/5/2 | | |
| IUGR | 3 | | |
| GA (wks) | | 30.4 (5.8) | 27.9 (23.3–42.0) |
| PMA (wks) | | 35.7 (6.5) | 36.5 (24.9–47.9) |
| PNA (days) | | 38 (30) | 27 (5–121) |
| Birth wt (g) | | 1,518 (884) | 1,115 (540–3850) |
| Current wt (g) | | 2,060 (1020) | 1,955 (700–4200) |
| Serum creatinine concn (μmol/liter) | | 52 (32) | 41 (22–164) |
| Ciprofloxacin treatment | | | |
| Duration (days) | | 5 (4) | 5 (1–17) |
| Dose (mg/dose) | | 18.9 (10.1) | 18.7 (4.5–40.0) |
| Dose (mg/kg/dose) | | 9.1 (1.6) | 9.7 (4.4–11.0) |
| Comedication | | | |
| Inotropic agents | 22 | | |
| Teicoplanin | 41 | | |
| Diuretics | 30 | | |
| Caffeine | 15 | | |
| Amoxicillin-clavulanic acid | 12 | | |
| Nystatin | 12 | | |
| Colistin-tobramycin-amphotericin B | 10 | | |

[a] IUGR, intrauterine growth restriction; GA, gestational age at birth; PMA, postmenstrual age; PNA, postnatal age.

**Model building.** For population modeling, 430 ciprofloxacin concentrations (265 pharmacokinetic and 165 scavenged samples) were available. The ciprofloxacin concentrations of pharmacokinetic and scavenged samples ranged from 450 to 15,976 and from 52 to 10,961 ng/ml, respectively. The concentration versus time profile is shown in Fig. 3.

A two-compartment model with first-order elimination fitted the data. The OFV value and residual variability of the two-compartment model were lower than those of the one-compartment

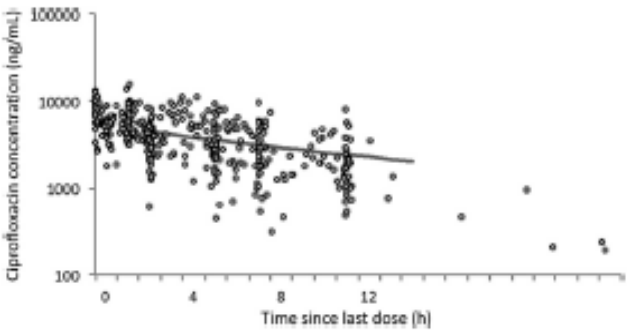


FIG 3 Ciprofloxacin concentrations versus time. The solid line represents the population prediction of a typical patient.

TABLE 3 Covariate analysis

| Characteristic[a] | Pharmacokinetic parameter(s) | Objective function value | IIV[b] CL (%) |
|---|---|---|---|
| Structural model | | **6,569.0**[c] | **98.3** |
| Allometric model | CL, $V_1$, $V_2$, Q | | |
| Current body wt | | **6,455.1** | **67.6** |
| Impact of age | V1 | | |
| GA | | 6,454.9 | |
| PNA | | 6,451.9 | |
| PMA | | 6,453.2 | |
| Impact of age | $V_2$ | | |
| GA | | 6,455.1 | |
| PNA | | 6,452.3 | |
| PMA | | 6,453.7 | |
| Impact of renal maturation | CL | | |
| Birth wt | | 6,442.0 | |
| GA | | 6,438.7 | |
| PNA | | 6,425.2 | |
| PMA | | 6,399.5 | 43.6 |
| Birth wt and PNA | | 6,396.2 | |
| GA and PNA | | **6,388.4** | **42.5** |
| Impact of renal function | CL | | |
| Serum creatinine | | **6,429.3** | |
| Impacts of renal maturation and renal function | CL | | |
| GA, PNA, and serum creatinine | | **6,371.3** | **36.9** |
| Impacts of renal maturation, renal function, and comedication | CL | | |
| GA, PNA, serum creatinine, and diuretics | | 6,371.4 | |
| GA, PNA, serum creatinine, and caffeine | | 6,369.2 | |
| GA, PNA, serum creatinine, and teicoplanin | | 6,371.0 | |
| GA, PNA, serum creatinine, and amoxicillin-clavulanic acid | | 6,366.8 | |
| GA, PNA, serum creatinine, and nystatin | | 6,370.9 | |
| GA, PNA, serum creatinine, and inotropic agents | | **6,363.8** | **34.5** |

[a] GA, gestational age; PNA, postnatal age; PMA, postmenstrual age.
[b] IIV, interindividual variability.
[c] OFV values of model with significant improvement are indicated in boldface.

model. The model was parameterized in terms of the central volume of distribution ($V_1$), the peripheral volume of distribution ($V_2$), the intercompartment clearance (Q), and the clearance (CL) of ciprofloxacin. Interindividual variability was best described by an exponential model and was then estimated for $V_1$, $V_2$, and CL. Interoccasion variability on CL was coupled to interindividual variability by an additive model, respectively. A proportional model best described residual variability.

**Covariate analysis.** The allometric size approach was used by incorporating *a priori* the current weight into the basic model (allometric coefficients of 0.75 for CL and Q and of 1 for $V_1$ and $V_2$), which caused a significant drop in the OFV of 113.9 points. Postmenstrual age was identified as the most important covariate on CL, associated with a drop in the OFV of 55.6 U. However, gestational age and postnatal age together proved to be superior (ΔOFV 66.7 U) to postmenstrual age alone. A further decrease in the OFV of 17.1 U was achieved by implementing the serum creatinine concentration on clearance. The model was further improved by introducing the coadministration of inotropic agents (ΔOFV 7.5 U) as a third covariate on clearance. For $V_1$, only the coadministration of inotropic agents caused a significant drop in the OFV of 3.9 points in the forward selection process. However, it was not retained in the model after the backward selection process. A detailed presentation of the covariate analysis results is presented in Table 3. Size explained 31.2%, renal maturation explained 25.6%, renal function explained 5.7%, and coadministration of inotropic agents explained 2.4% of the ciprofloxacin CL variability. The η shrinkages were 5.4% for CL, 25.7% for $V_1$, and 25.0% for $V_2$, respectively. The ε shrinkage was 13.6%.

Table 4 summarizes parameter estimates of the final pharmacokinetic model. The median (range) of estimated weight-normalized CL and volume distribution at steady state (sum of $V_1$ and $V_2$) were 0.20 (0.04 to 0.81) liters/h/kg and 2.02 (0.40 to 3.55) liters/kg, respectively. The $AUC_{0-24}$ at steady state for the evaluated dose regimen ranged from 35 to 291 mg-h/liter. Ciprofloxacin CL increased allometrically with current weight in neonates and young infants, decreased with increasing creatinine concentration, and showed a 29% decrease with the coadministration of inotropic agents. The relationship between ciprofloxacin weight-normalized CL (liters/kg) versus postmenstrual age is shown in Fig. 4.

TABLE 4 Population pharmacokinetic parameters of ciprofloxacin and bootstrap results

| Parameter[a] | Full data set | | Bootstrap | |
|---|---|---|---|---|
| | Final estimate | RSE (%) | Median | 5th–95th percentile |
| $V_1$ (liters) | | | | |
| $V_1 = \theta1 \times (CW/1955)$ | | | | |
| θ1 | 1.97 | 17.7 | 1.82 | 0.78–2.59 |
| $V_2$ (liters) | | | | |
| $V_2 = \theta2 \times (CW/1955)$ | | | | |
| θ2 | 1.93 | 21.9 | 1.97 | 1.38–3.02 |
| Q (liters/h) | | | | |
| $Q = \theta3 \times (CW/1955)^{0.75}$ | | | | |
| θ3 | 2.5 | 32.6 | 2.62 | 1.02–5.41 |
| CL (liters/h) | | | | |
| $CL = \theta4 \times (CW/1955)^{0.75} \times F_{age} \times RF \times F_{inotrope}$ | | | | |
| θ4 | 0.366 | 6.0 | 0.365 | 0.323–0.407 |
| $F_{age} = (GA/27.9)^{\theta5} \times (PNA/27)^{\theta6}$ | | | | |
| θ5 | 2.11 | 11.9 | 2.09 | 1.60–2.57 |
| θ6 | 0.494 | 10.8 | 0.492 | 0.386–0.606 |
| $RF = EXP[(CREA-42) \times \theta7]$ | | | | |
| θ7 | −0.00335 | 46.0 | −0.00331 | −0.00753 to −0.00063 |
| $F_{inotrope}$ | | | | |
| θ8 | 0.708 | 10.9 | 0.719 | 0.572–0.869 |
| Interindividual variability (%) | | | | |
| $V_1$ | 48.1 | 63.6 | 49.6 | 26.2–77.2 |
| $V_2$ | 49.3 | 68.3 | 51.2 | 15.8–76.9 |
| CL | 33.2 | 19.9 | 31.3 | 25.3–37.4 |
| Interoccasion variability (%) | | | | |
| CL | 16.4 | 55.6 | 16.6 | 9.2–26.2 |
| Residual variability (%) | 19.3 | 28.2 | 18.7 | 14.8–23.1 |

[a] $V_1$, central volume of distribution; $V_2$, peripheral volume of distribution; Q, intercompartment clearance; CL, clearance; RF, renal function; CW, current weight in grams; $F_{inotrope}$, scaling factor applied for patients coadministered with inotropic agents; CREA, serum creatinine concentration in μmol/liter; GA, gestational age in weeks; PNA, postnatal age in days. In our population, 1,955 g, 27.9 weeks, 27 days, and 42 μmol/liter were the median current weight (at the day of the study), gestational age, postnatal age, and serum creatinine concentration values, respectively.

**Model evaluation.** Model diagnostics showed acceptable goodness-of-fit for the final model of ciprofloxacin. As shown in Fig. 5A and B, the predictions are unbiased. In the diagnostic plots of CWRES versus time and PRED, no trends were observed (Fig. 5C and D). In addition, the median parameter estimates resulting from the bootstrap procedure closely agreed with the respective values from the final population model, indicating that the final model is stable and can redetermine the estimates of population pharmacokinetic parameters (Table 4). The NPDEs are presented in Fig. 5E and F. NPDE distribution and histogram met well the theoretical N(0,1) distribution and density, indicating a good fit of the model to the individual data. The mean and variance of the NPDE were 0.05 (Wilcoxon signed-rank test, $P = 0.19$) and 0.91 (Fisher variance test, 0.17), respectively. The pcVPC is shown in Fig. 5G. The prediction-corrected observed concentrations fit well the simulated concentrations, confirming the predictive performance of the developed model.

**Ciprofloxacin penetration into the CSF.** The concentrations of ciprofloxacin in six CSF samples ranged from 187 to 1,650 ng/ml, respectively. The median value of CSF/serum concentration ratio was 0.32 (range, 0.08 to 0.58). A trend for correlation between the CSF collection time and the CSF/serum concentration ratios was demonstrated, suggesting less elimination from (or diffusion in) the CSF than the systemic circulation (Fig. 6).

**Dosing regimen optimization.** The target attainment rates as a function of dose and age groups for a standard MIC susceptibility breakpoint of 0.5 mg/liter are shown in Fig. 7. A cutoff point of a PMA of 34 weeks was selected to separate age groups based on visual inspection of the plot showing CL versus PMA (Fig. 4).

A total of 90% of simulated newborns with a PMA of <34 weeks and 84% of newborns with a PMA of ≥34 weeks achieved the target AUC/MIC values of 125 h when treated at doses of 7.5 and 12.5 mg/kg BID, respectively. The associated risks of overdose (AUC > 291 mg·h/liter, the maximal value reported in the present study, did not show short-term adverse events) for the proposed dosing regimen were <8%. Higher doses will be required for patients with more resistant bacterial strains.

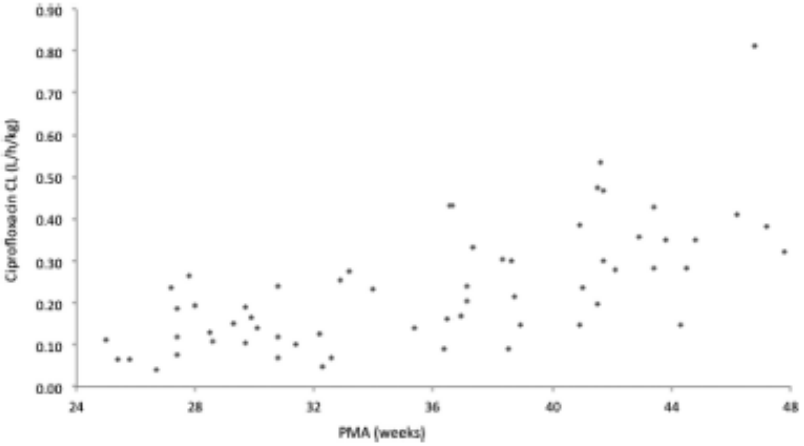


FIG 4 Ciprofloxacin CL versus PMA.

## DISCUSSION

This is the first population pharmacokinetic study of ciprofloxacin conducted in a cohort of neonates and young infants. It was undertaken to estimate ciprofloxacin pharmacokinetic parameters and to evaluate the impact of demographic, clinical, and biological factors on ciprofloxacin disposition. Our results show that a two-compartment model with first-order elimination with gestational age, postnatal age, current weight, serum creatinine concentration, and coadministration of inotropic agents was optimal for data modeling.

Ciprofloxacin is a fluoroquinolone targeting bacterial DNA gyrase enzyme, a member of the class of type II topoisomerases that displays *in vitro* activity against most Gram-negative and many Gram-positive pathogenic bacteria, many of which are resistant to a wide range of antibiotics. Ciprofloxacin has a complex safety profile. A range of adverse drug reactions, including arthropathies, without identified long-term effects, need to be balanced with its efficacy (14–16). Potential neuroprotective effects were demonstrated in a juvenile animal model subjected to *Escherichia coli* sepsis (17). Increase in multidrug-resistant sepsis forces the use of ciprofloxacin (18, 19). However, dosages vary widely from one country to another and from one center to another within the same country (4), since only a few pharmacokinetic studies have been conducted in preterm and term neonates (20–22). Since ciprofloxacin is mainly eliminated by the renal route, renal anatomical and functional maturation is expected to have a major influence on ciprofloxacin clearance and dosing in neonates and young infants. When the current weight is taken into account using an allometric scaling approach, the independent impact of gestational and postnatal ages on clearance illustrates the influence of both antenatal and postnatal renal maturation on ciprofloxacin clearance (Fig. 8); this includes the expected consequences of the normal pattern of renal function ontogeny (23).

Renal function, as reflected by serum creatinine concentrations, was significantly and independently correlated with ciprofloxacin clearance in the present study. The relation of serum creatinine to the glomerular filtration rate (GFR) at birth is complicated. There was no unique equation to describe the relationship between renal function and drug clearance in neonates. We have demonstrated that the variation in the serum creatinine measurement had a considerable impact on the transferability of a published creatinine-based dosing regimen of renal excreted drug to different clinical settings (24). The influence of residual maternally derived creatinine seems to be limited in our study, since neonates at ≥5 days of age were included. Ciprofloxacin clearance decreased with the coadministration of inotropic drugs. A possible explanation is that inotropic agents are often given when there is decreased blood pressure, which will result in decreased GFR. The same effect was observed by Seay et al. (25), who reported that the coadministration of dopamine induced a 28% decrease in vancomycin clearance in neonates. The use of inotropic agents may be also a surrogate for underlying hemodynamic instability and altered renal hemodynamics, resulting in decreased drug elimination, as suggested by previous investigators (25). We were unable to demonstrate any significant effect of ventilation or intrauterine growth retardation on ciprofloxacin disposition. Clearly, the impact of these variables on ciprofloxacin neonatal pharmacokinetics is complex, probably even more in critically ill neonates. In addition, our study is probably not able to demonstrate their full impact.

In adults, the best surrogate for the ciprofloxacin pharmacokinetic-pharmacodynamic relationship is the $AUC_{0-24}$/MIC ratio. A target $AUC_{0-24}$/MIC value of 125 h was required against Gram-negative infections (6, 7). According to regulatory guidelines (26–28), the pharmacokinetic-pharmacodynamic relationship for most anti-infective drugs can be assumed to be similar across all age groups, including neonates, making ciprofloxacin a good example for demonstrating that modeling and simulation approaches can be used to establish optimal dosage recommendations in neonates. Dosage administration in neonates is commonly based on an mg/kg basis according to the different age groups (preterm or term neonates), and a standard mg/kg dose is calculated accordingly in each group. In the present study and in order to reach "optimal but practical dosage recommendations" for clinicians, two groups of patients were defined, newborns with a PMA of <34 weeks or a PMA of ≥34 weeks, and the dose required to achieve the predefined AUC/MIC target was adapted to body weight (i.e., in mg/kg) in the two age groups. The simulation approach demonstrates that doses of 7.5 mg/kg for new-

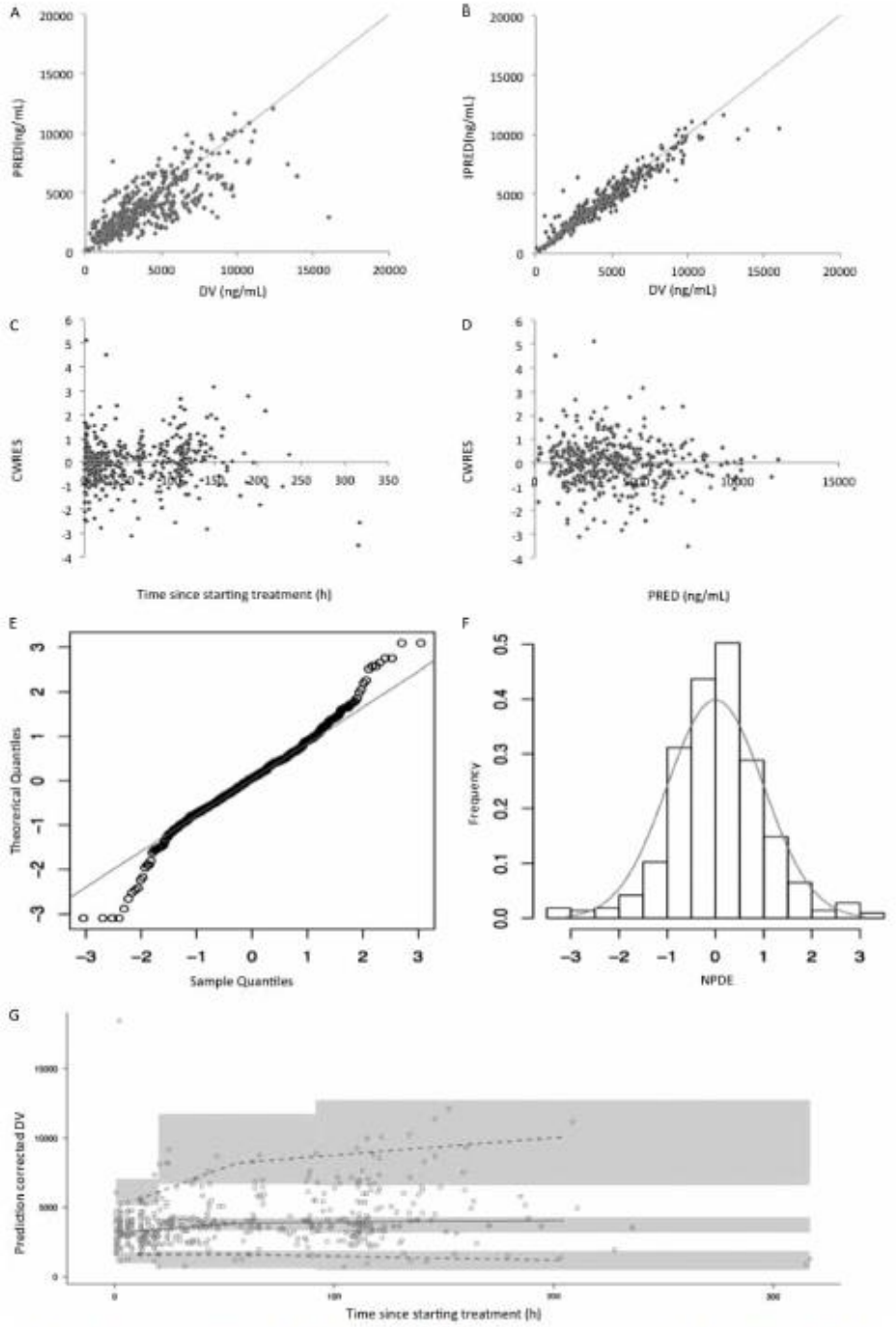


FIG 5 Model evaluation for ciprofloxacin. (A) Population predicted (PRED) versus observed concentrations (DV); (B) individual predicted (IPRED) versus DV; (C) conditional weighted residuals (CWRES) versus time; (D) CWRES versus PRED; (E) QQ-plot of the distribution of the normalized prediction distribution errors (NPDE) versus the theoretical N(0,1) distribution; (F) histogram of the distribution of the NPDE, with the density of the standard Gaussian distribution overlaid; (G) prediction-corrected visual predictive check. The circles represent the prediction-corrected observed concentrations. The solid line represent the median prediction-corrected observed concentrations and semitransparent gray field represents simulation-based 95% confidence intervals for the median. The observed 5th and 95th percentiles are indicated by dashed lines, and the 95% intervals for the model-predicted percentiles are indicated as corresponding semitransparent gray fields.

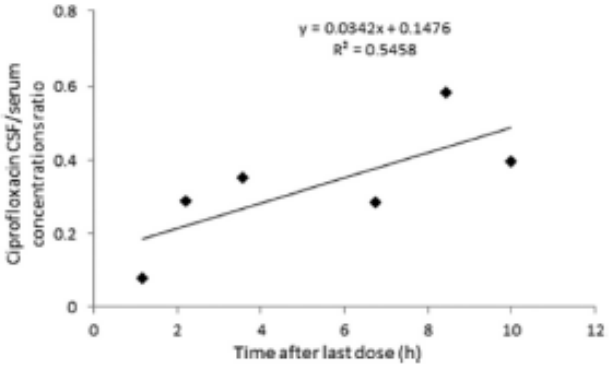


FIG 6 Ciprofloxacin CSF/serum concentration ratio versus time.

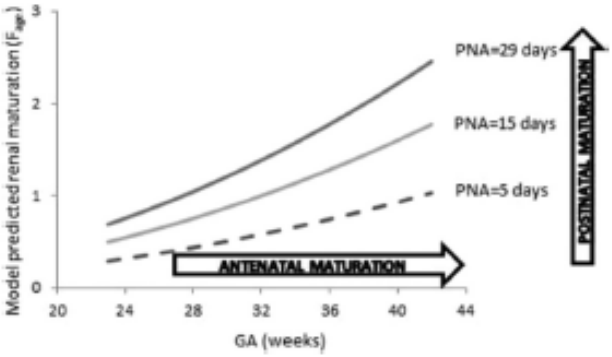


FIG 8 Model-based predicted impact of renal maturation on the clearance of ciprofloxacin in neonates. The influence of both gestational age (GA; representing antenatal maturation) and postnatal age (PNA; representing postnatal maturation) on renal maturation is depicted graphically.

borns with a PMA of <34 weeks and 12.5 mg/kg for newborns with a PMA of ≥34 weeks given twice daily allowed 90 and 84% of the patients, respectively, to achieve the AUC/MIC target, with a standard EUCAST MIC susceptibility breakpoint of 0.5 mg/liter (29). For this optimal dosing regimen, the associated risks of overdose, which was defined as the simulated AUC over the maximum reported value of 291 mg·h/liter, were low (<8%), which supports a balanced efficacy and safety profile associated with the recommended dosing regimens. Clearly, organisms with a higher MIC would require a higher AUC to achieve the same outcome (6, 30). In the United States, the Clinical Laboratory Standards Institute's breakpoint is even higher with a, MIC of >1 mg/liter (31) and, as a consequence, a double dose (with assumption of linear pharmacokinetics) would be required to achieve the same AUC/MIC target.

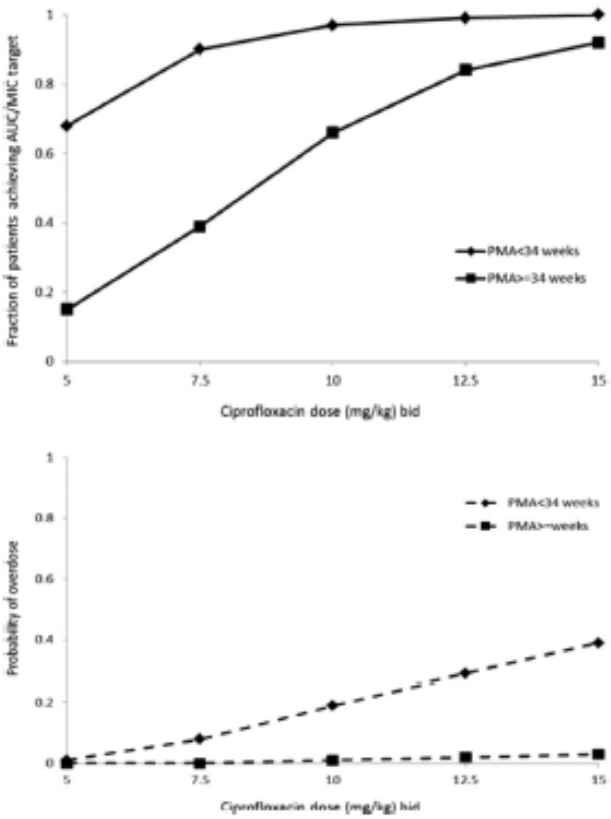


FIG 7 Target attainment rates and probability of overdose. The target attainment rates for the 100 simulated trials for MIC value of 0.5 mg/liter is presented as a function of the dose and age group. The AUC/MIC target is 125. Overdose is defined as an AUC over the maximum reported value of 291 mg·h/liter.

The pharmacokinetic model of ciprofloxacin was developed and internally validated. External validation was not performed because of the limited number of patients currently exposed to this drug. Ultimately, a patient-tailored dose based on modeling and simulation has to be evaluated in clinical practice to confirm its clinical benefits.

**Conclusion.** A population pharmacokinetic model of ciprofloxacin was developed in neonates and young infants, showing a low clearance of the drug, compared to older children and adults. Gestational age at birth, postnatal age, current weight, serum creatinine concentration, and the coadministration of inotropic agents had significant impact on ciprofloxacin pharmacokinetics. The ciprofloxacin dosing regimen in neonates and young infants <3 months old was established based on population pharmacokinetics analysis.

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E.J.A., C.L.G., B.K., J.N.V.D.A., and M.A.T. designed the study; all authors contributed to the final version of the protocol; H.H., W.Z., C.L.G., and E.J.-A. developed the eCRF, H.H. designed S.P., H.H., T.N., S.M., S.P., and M.A.T. organized the trial and recruited patients; W.Z. and E.J.-A. set up the analytical method; W.Z., C.L.G., and E.J.-A. performed the population pharmacokinetic analysis; W.Z. and E.J.-A. drafted the first version of the manuscript; significant contributions to the final version were provided by C.L.G., J.N.V.D.A., and G.L.K.

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ORIGINAL RESEARCH ARTICLE

# Pharmacokinetic Studies in Neonates: The Utility of an Opportunistic Sampling Design

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**Abstract**

*Background and Objective* The use of an opportunistic (also called scavenged) sampling strategy in a prospective pharmacokinetic study combined with population pharmacokinetic modelling has been proposed as an alternative strategy to conventional methods for accomplishing pharmacokinetic studies in neonates. However, the reliability of this approach in this particular paediatric population has not been evaluated. The objective of the present study was to evaluate the performance of an opportunistic sampling strategy for a population pharmacokinetic estimation, as well as dose prediction, and compare this strategy with a predetermined pharmacokinetic sampling approach.

*Methods* Three population pharmacokinetic models were derived for ciprofloxacin from opportunistic blood samples (SC model), predetermined (i.e. scheduled) samples (TR model) and all samples (full model used to previously characterize ciprofloxacin pharmacokinetics), using NONMEM software. The predictive performance of developed models was evaluated in an independent group of patients.

*Results* Pharmacokinetic data from 60 newborns were obtained with a total of 430 samples available for analysis; 265 collected at predetermined times and 165 that were scavenged from those obtained as part of clinical care. All datasets were fit using a two-compartment model with first-order elimination. The SC model could identify the most

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significant covariates and provided reasonable estimates of population pharmacokinetic parameters (clearance and steady-state volume of distribution) compared with the TR and full models. Their predictive performances were further confirmed in an external validation by Bayesian estimation, and showed similar results. Monte Carlo simulation based on area under the concentration–time curve from zero to 24 h ($AUC_{24}$)/minimum inhibitory concentration (MIC) using either the SC or the TR model gave similar dose prediction for ciprofloxacin.

*Conclusion* Blood samples scavenged in the course of caring for neonates can be used to estimate ciprofloxacin pharmacokinetic parameters and therapeutic dose requirements.

**Key Points**

The challenges associated with performing pharmacokinetic studies in sick neonates can be mitigated using residual blood samples from specimens required for routine medical care.

A population pharmacokinetic model based on opportunistic sampling strategy could identify the most significant covariates and provide reasonable estimates of clearance and volume of distribution compared with the standard predetermined sampling strategy.

The generalizability of a population pharmacokinetic study using an opportunistic sampling approach depends on the density and quality of sampling, as well as the stability of the drug under evaluation.

The resources required to conduct an opportunistic pharmacokinetic study are the same as other designs. In order to reduce the period of neonatal exposure to potentially non-adapted dosage schedules, mixing predetermined (sparse) pharmacokinetic and opportunistic samples may be an optimal option for determining pharmacokinetic parameters of drugs under evaluation in neonates.

## 1 Introduction

A thorough understanding of developmental pharmacokinetics is essential for optimizing drug therapy in neonates. Challenges in obtaining pharmacokinetic data in neonates include frequent restriction in vascular access and both the number and volume of blood samples that can be obtained, difficulty in precise timing of samples consequent to the demands of caring for a sick neonate, and a relatively low rate of informed parental consent for interventions considered by some to be nontherapeutic [1]. Some scientific attempts have been made to increase the feasibility of pharmacokinetic studies in neonates. One of the novel proposed methods is the use of opportunistic samples (samples collected from blood remaining after routine laboratory tests as part of clinical care) combined with population pharmacokinetic analysis. While this approach has been applied to the evaluation of piperacillin and metronidazole pharmacokinetics in preterm infants [1, 2], previous studies have not compared the population pharmacokinetic parameters (typical values and variability) with those derived from traditional predetermined sampling strategies. Comparison of predictive performances of these approaches on dose prediction has been difficult due to the relatively few predetermined pharmacokinetic samples that were obtained (4 % of the total number of samples in the piperacillin study and 10 % in metronidazole study) [1, 2].

In a recent study of ciprofloxacin pharmacokinetics in neonates conducted within the EU framework 7 GRIP (Global Research in Paediatrics) and TINN (Treat Infections in NeoNates) projects, we reported the pharmacokinetics of ciprofloxacin from a cohort of neonates enrolled in a prospective, population-based pharmacokinetic study [3]. In the context of this study, opportunistic blood samples (i.e. those obtained as part of clinical patient care) were also available. This enabled us to conduct a companion investigation to specifically evaluate the performance of an opportunistic sampling strategy in pharmacokinetic parameter estimation via comparison of this approach with a traditional strategy using predetermined pharmacokinetic sampling. The results of this study are reported herein.

## 2 Methods

### 2.1 Pharmacokinetic Data

The pharmacokinetic data were obtained in the context of a prospective, open-label pharmacokinetic study of ciprofloxacin, conducted in the neonatal intensive care unit in the Liverpool Women's Hospital and the paediatric intensive care unit in the Alder Hey Children's Hospital, Liverpool, UK. All parents were informed about the two different methods of sampling. The study design and inclusion criteria have been previously reported [3]. Briefly, neonates and young infants <3 months of age who received ciprofloxacin therapy as part of their clinical care were studied. Ciprofloxacin (generic, Peckforton Pharmaceuticals, Crewe, UK) was administered as an intravenous infusion over 30–60 min by a syringe pump connected to

microbore tubing. Neonates with a postmenstrual age (PMA) of <40 weeks received 10 mg/kg twice daily (every 12 h), whereas young infants with a PMA ≥40 weeks were treated with 10 mg/kg twice or three times daily.

### 2.2 Pharmacokinetic Sampling

Two types of pharmacokinetic samples were available during the ciprofloxacin treatment period:

- Predetermined pharmacokinetic samples (TR): A three-timepoint schedule was designed to cover the full pharmacokinetic profiles of ciprofloxacin. Patients were equally assigned to one of the two predefined timed sampling groups, as shown in Table 1. These samples were collected on day 1 or day 2 of treatment, and again between day 5 and day 7. This pharmacokinetic schedule required a maximum of 1.2 mL of blood, which was consistent with the European Medicines Agency (EMA), who recommended a maximum of 1 % of blood loss on each occasion and 3 % over 4 weeks.
- Opportunistic (scavenged) sampling (SC): During the whole ciprofloxacin treatment period, opportunistic samples were collected from blood remaining after routine biochemical tests, which were ordered by physicians. No additional blood volume was taken for these samples. Tubes with specific research labels were used, allowing the laboratory staff to identify the opportunistic samples and store them at −70 °C after routine test. The standard operation procedure to handle the opportunistic samples is the same as the predetermined pharmacokinetic samples. Precise sampling time and drug administration history (including dosing and infusion time) were recorded prospectively by the clinical team using dedicated documentation supervised by a research nurse and transcribed later to the electronic case report form. Samples without precise sampling time were excluded. All blood samples were refrigerated and then centrifuged. Plasma was removed and stored at −70 °C. The delay between sampling and storage was <24 h. Samples were shipped on dry ice to the Department of Paediatric Pharmacology, Robert Debré Hospital, where they were stored at −70 °C prior to analysis, a period not exceeding 12 months from the time of collection.

**Table 1** Predetermined pharmacokinetic sampling schedule

| Group | Sampling times[a] | | | |
|---|---|---|---|---|
| Ciprofloxacin administered twice daily | | | | |
| A | End of infusion[b] | T3 | T8 | |
| B | T2 | T6 | T12 | |
| Ciprofloxacin administered three times daily | | | | |
| C | End of infusion[b] | T3 | T8 | |
| D | T2 | T4 | T8 | |

[a] Babies weighing <1000 g will have samples taken randomly at two specific times to minimize blood loss and to ensure representation of each period

[b] The sampling times are calculated from the start of infusion of ciprofloxacin

### 2.3 Analytical Method of Ciprofloxacin

The analytical method for quantitation of ciprofloxacin has been previously reported [4]. Briefly, ciprofloxacin concentrations were determined using high-performance liquid chromatography combined with mass spectrometry using ciprofloxacin-$d_8$ as the internal standard. The calibration curve ranged from 25 to 3000 ng/mL. The inter- and intraday coefficients of variation (CVs) of controls were 4.1 and 2.4 %, respectively, and the lower limit of quantification was 25 ng/mL. Only 50 µL plasma or serum was required for quantification. The short- and long-term stabilities of ciprofloxacin in plasma and serum were documented for at least 24 h and 12 months, respectively.

### 2.4 Evaluation of the Opportunistic Sampling Strategy

#### *2.4.1 Population Pharmacokinetic Modelling*

Three population pharmacokinetic models were developed: an opportunistic sampling model (SC model) derived from opportunistic samples, a pharmacokinetic sampling model (TR model) derived from predetermined pharmacokinetic samples, and a full sampling model (full model) derived from all samples. The performance of the SC model was compared with the TR and full models in terms of covariate analysis, variability, typical pharmacokinetic parameters estimation, and model evaluation.

Pharmacokinetic analyses were carried out using the nonlinear mixed effects modelling program NONMEM V 7.2 (Icon Development Solutions, San Antonio, Texas, USA). The same modelling and validation process was used for all three models.

Different one- or two-compartment open models with first- or zero-order elimination were compared. The basic model was evaluated through visual inspection of routine diagnostic plots. The first-order conditional estimation (FOCE) method with an interaction option was used to estimate pharmacokinetic parameters and their variability. In each case, interindividual variability (IIV) of the pharmacokinetic parameters was estimated using an exponential model, and could be expressed as follows:

$$\theta_i = \theta_{\text{mean}} \times e^{\eta_i}$$

where $\theta_i$ represents the parameter value of the $i$th subject, $\theta_{\text{mean}}$ represents the typical value of the parameter in the

population, and $\eta i$ represents the variability between subjects, which is assumed to follow a normal distribution with a mean of zero and variance of $\sigma^2$.

Covariate analysis followed a forward and backward selection process. The likelihood ratio test was used to test the effect of each variable on model parameters. The effects of current weight, birth weight, gestational age, postnatal age, PMA, serum creatinine concentration (collected within 48 h of pharmacokinetic sampling), and comedication of inotropic agents, teicoplanin, diuretics, amoxicillin-clavulanic acid or nystatin [evaluated separately as categorical covariates and preselected according to the potential impact on pharmacokinetics (e.g. nephrotoxicity, surrogate of disease) and frequency of use] were investigated as potential variables affecting pharmacokinetic parameters. During the first step of covariate model building, a covariate was included if a significant ($p < 0.05$; Chi-square distribution with one degree of freedom) decrease (reduction > 3.84) in the objective function value (OFV) from the basic model was obtained. All the significant covariates were then added simultaneously into a 'full' model. Subsequently, each covariate was independently removed from the full model. If the increase in the OFV was higher than 6.635 ($p < 0.01$; Chi-square distribution), the covariate was considered as significantly correlated with the pharmacokinetic parameter, and was therefore retained in the final model.

Model validation was based on graphical and statistical criteria. Goodness-of-fit plots, including observed (DV) versus population prediction (PRED); DV versus individual prediction (IPRED); conditional weighted residuals (CWRES) versus time and CWRES versus PRED were initially used for diagnostic purposes [5]. The stability and performance of the final models were also assessed by a nonparametric bootstrap with re-sampling and replacement. Re-sampling was repeated 500 times, and the values of estimated parameters from the bootstrap procedure were compared with those estimated from the original dataset. The entire procedure was performed in an automated fashion, using Perl-speaks-NONMEM (PsN), version 2.30 [6]. The final models were also evaluated both graphically and statistically by normalized prediction distribution errors (NPDE) [7]. Overall, 1000 datasets were simulated using the final population model parameters. NPDE results were summarized graphically by default, as provided by the NPDE R package, version 1.2 [8]: (1) QQ-plot of the NPDE; (2) histogram of the NPDE. The NPDE is expected to follow the $N$ (0, 1) distribution.

#### 2.4.2 *External Evaluation*

The predictive performance of developed models was further evaluated in an independent group of neonates and young infants ($n = 14$, number of samples = 23). The individual concentrations were predicted by Bayesian estimation ('MAXEVAL = 0' in the ESTIMATION step) with NONMEM using the population pharmacokinetic parameters. The predictive performance was evaluated by calculating the prediction error (PE) using the following equation:

$$\text{PE}\ (\%) = (\text{individual predicted concentration} - \text{measured concentration})/\text{measured concentration}$$

#### 2.4.3 *Dose Prediction*

Given our interest in dose prediction using model-based approaches, the predictive performance of the opportunistic sampling strategy was evaluated via simulation. Target systemic exposure [expressed as the area under the plasma concentration vs. time curve (AUC)] attainment rates in neonates were used as the endpoint for the evaluation as AUC appears to be the best predictor of microbiological and clinical outcome of ciprofloxacin. A ciprofloxacin target AUC from time zero to 24 h ($AUC_{24}$)/minimum inhibitory concentration (MIC) value of 125 h was used as a pharmacodynamic surrogate predictor of microbiological and clinical outcome for Gram-negative infections [9, 10] and the basis of comparing the respective sampling strategies.

Monte Carlo simulations were performed with the pharmacokinetic parameter estimates obtained from the SC and TR models. A milligram per kilogram dose of ciprofloxacin representing various dose levels (e.g. 5, 7.5, 10, 12.5 and 15 mg/kg every 12 h) was evaluated in the simulated patients. A total of 100 simulations were performed from each model, and $AUC_{24}$ at steady state was calculated for each simulated patient.

## 3 Results

### 3.1 Study Population and Ciprofloxacin Concentrations

Pharmacokinetic data from 60 neonates and young infants were obtained from the TINN ciprofloxacin study [3]. Of these, seven received ciprofloxacin at 5 mg/kg/dose twice daily, six were administered ciprofloxacin at 10 mg/kg/dose three times daily, and 47 were administered ciprofloxacin at 10 mg/kg/dose twice daily. The treatment lasted for a median of 5 days (range 1–17 days). A summary of patient characteristics is presented in Table 2. All covariates were normally distributed. Three pharmacokinetic datasets were constructed from the original dataset: 165 opportunistic samples for the SC model, 265 specific

**Table 2** Baseline characteristics in 60 neonates and young infants

| | Number | Mean (SD) | Median (range) |
|---|---|---|---|
| Patients | 60 | | |
| Sex (male/female) | 39/21F | | |
| Race | 53 Caucasian/5 Asian/2 unknown | | |
| GA (weeks) | | 30.4 (5.8) | 27.9 (23.3–42.0) |
| PMA (weeks) | | 35.7 (6.5) | 36.5 (24.9–47.9) |
| PNA (days) | | 38 (30) | 27 (5–121) |
| Birth weight (g) | | 1518 (884) | 1115 (540–3850) |
| Current weight (g) | | 2060 (1020) | 1955 (700–4200) |
| Serum creatinine concentration (μmol/L) | | 52 (32) | 41 (22–164) |
| Ciprofloxacin dose (mg/dose) | | 18.9 (10.1) | 18.7 (4.5–40.0) |
| Ciprofloxacin dose (mg/kg/dose) | | 9.1 (1.6) | 9.7 (4.4–11.0) |
| Coadministration of inotropic agents | 22 | | |
| Pharmacokinetic data | | | |
| Opportunistic samples | 165 | | |
| Samples per patient[a] | | 2.73 (2.11) | 2 (0–10) |
| Concentrations (ng/mL) | | 4257 (2517) | 4066 (52–10,961) |
| Predetermined samples | 265 | | |
| Samples per patient | | 4.38 (1.49) | 4 (2–6) |
| Concentrations (ng/mL) | | 4120 (2660) | 3505 (450–15,976) |

*GA* gestational age at birth, *PMA* postmenstrual age (sum of GA and PNA), *PNA* postnatal age, *SD* standard deviation

[a] Fifty-five of the 60 neonates provided the opportunistic samples

pharmacokinetic samples for the TR model, and all 430 samples for the full model.

The measured ciprofloxacin concentrations ranged from 52 to 10,961 (median 4066; 25th–75th: 2212–5748) ng/mL for opportunistic samples and 450 to 15,976 (median 3505; 25th–75th: 2191–5428) for specific pharmacokinetic samples, respectively. The concentration versus time profiles are shown in Fig. 1.

### 3.2 Evaluation of Opportunistic Sampling Strategy in Population Modelling

#### *3.2.1 Structural Model and Covariate Analysis*

All three datasets fitted two-compartment models with first-order elimination. The models were parameterized in terms of central volumes of distribution ($V_1$), peripheral volume of distribution ($V_2$), intercompartment clearance ($Q$) and total plasma clearance of ciprofloxacin (CL).

The allometric size approach, which consists of a priori incorporation of the current weight into the structural models, caused the most significant drops in the OFV (113.9 for the full model, 113.4 for the TR model, and 89.1 for the SC model) for all three models. The clearance and volume of distribution of ciprofloxacin increased allometrically with current weight in neonates and young infants. After incorporation of the current patient weight, PMA was identified as the most important covariate of CL, causing a drop in the OFV of 55.6 for the full model, 52.0 for the TR model, and 56.2 for the SC model. However, gestational age and postnatal age together always proved to be superior (ΔOFV 66.7 for the full model, 60.7 for the TR model, and 66.1 for the SC model) to PMA alone. A further decrease in the OFV was achieved by adding the effect of serum creatinine on clearance (ΔOFV 17.1 for the full model, 20.9 for the TR model, and 15.7 for the SC model). In particular, a reduction of clearance was observed with increasing serum creatinine concentration. The models were further improved by introducing coadministration of inotropic agents (ΔOFV 7.5 for the full model, 5.3 for the TR model, and 4.1 for the SC model) as a third covariate on clearance in the forward selection process, but this covariate was not retained after the backward selection process in the TR and SC models as it was not statistically significant. The extent of the decrease of ciprofloxacin clearance with coadministration of inotropic agents was 29 % for the full model, 24 % for the TR model, and 21 % for the SC model. Covariate analysis results are summarized in Table 3.

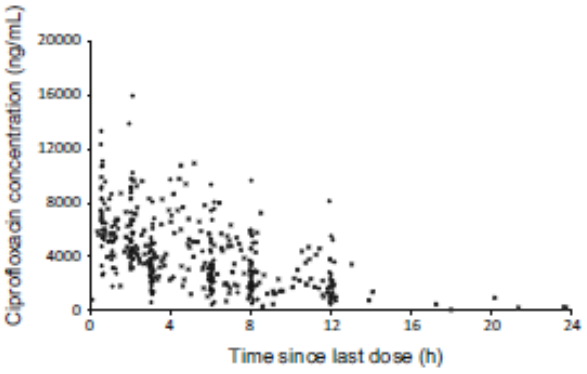


**Fig. 1** Ciprofloxacin concentrations versus time. *Diamonds* represent special pharmacokinetic samples, and *crosses* represent opportunistic samples

### 3.2.2 Interindividual, Interoccasion and Residual Variability

For all three models, IIV was best described by an exponential model. IIV could be estimated for $V_1$, $V_2$ and CL in the full and TR models, but in the SC model it could only be estimated for $V_2$ and CL. IIV of CL was larger in the SC model (37.1 %) compared with the full (33.2 %) and TR (35.8 %) models.

Only the full model allowed the estimation of interoccasion variability on CL, which was coupled to IIV by an additive model. For all three models, residual variability (RV) was best described by a proportional model, and was found to be similar among the three models evaluated (19.3 % for the full model, 19.2 for the TR model, and 18.2 for the SC model).

### 3.2.3 Model Evaluation

Model diagnostics showed acceptable goodness-of-fit for all three models. Diagnostic plots are presented in Figs. 2 and 3 for the SC and TR models, respectively (plots for the full model have been previously published [3]). As shown in Figs. 2a, b and 3a, b, the predictions were unbiased. In the diagnostic plots of CWRES versus time and PRED, no trends were observed (Figs. 2c, d, and 3c, d). In addition, the median parameter estimates resulting from the bootstrap procedures closely agreed with the respective values from the final population models, indicating that the final model is stable and can re-determine the estimates of the population pharmacokinetic parameters (Table 4). The NPDEs are presented in Figs. 2e, f, and 3e, f. NPDE distributions and histograms met well the theoretical $N$ (0, 1) distribution and density, indicating a good fit of the models to the individual data. The mean and variance of NPDE were 0.05 (Wilcoxon signed rank test $p = 0.19$) and 0.91 (Fisher variance test 0.17), respectively, for the full model; 0.09 (Wilcoxon signed rank test $p = 0.40$) and 1.11 (Fisher variance test 0.32), respectively, for the SC model; and 0.07 (Wilcoxon signed rank test $p = 0.17$) and 0.95 (Fisher variance test 0.56), respectively, for the TR model. For model evaluation, the SC model showed similar performance in terms of goodness-of-fit, stability, and predictive ability compared with both the TR and full models.

### 3.2.4 Population Pharmacokinetic Parameter Estimation

Population pharmacokinetic parameters estimated from three models are displayed in Table 4. For CL, the typical estimated values were similar between each of the models

**Table 3** Covariate analysis

| Characteristic | PK parameter(s) | PK opportunistic sampling | | Predetermined PK sampling | | Full PK sampling | |
|---|---|---|---|---|---|---|---|
| | | Drops in the OFV | IIV CL (%) | Drops in the OFV | IIV CL (%) | Drops in the OFV | IIV CL (%) |
| Structural model | | – | 94.9 | – | 98.1 | – | 98.3 |
| Allometric model | CL, $V_1$, $V_2$, $Q$ | −89.1 | 64.3 | −113.4 | 68.9 | −113.9 | 67.6 |
| Impact of renal maturation GA and PNA | CL | −66.1 | 39.4 | −60.7 | 43.7 | −66.7 | 42.5 |
| Impact of renal maturation and renal function GA, PNA and serum creatinine | CL | −15.7 | 37.0 | −20.9 | 35.8 | −17.1 | 36.9 |
| Impact of renal maturation, renal function and comedication | CL | – | – | – | – | −7.5 | 34.5 |
| GA, PNA, serum creatinine and coadministration of inotropic agents | | | | | | | |

*GA* gestational age, *PNA* postnatal age, *IIV* interindividual variability, *OFV* objective function value presented for covariates allowing significant improvement, *PK* pharmacokinetic, *CL* clearance, $Q$ intercompartment clearance, $V_1$ central volume of distribution, $V_2$ peripheral volume of distribution

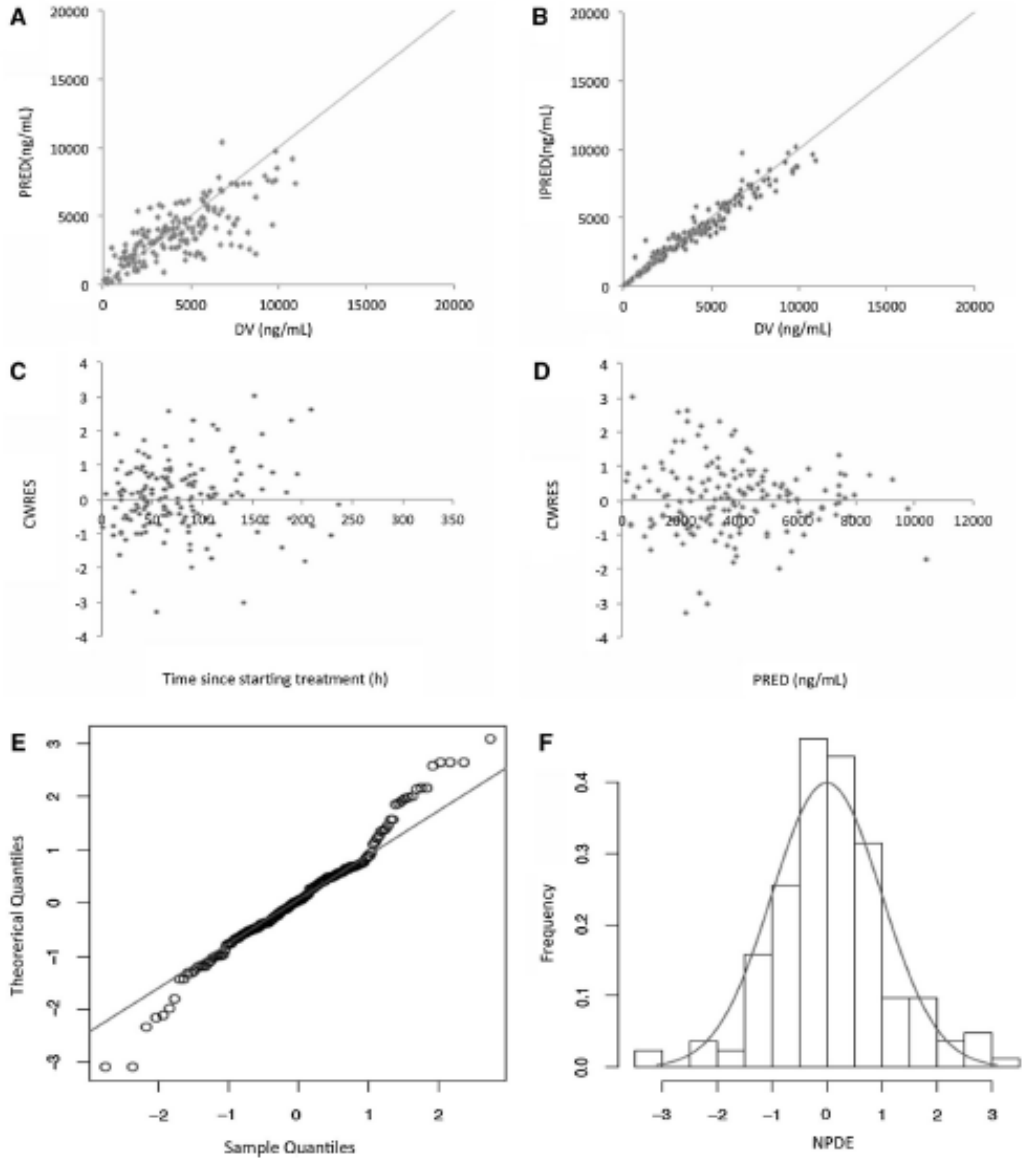


**Fig. 2** SC model evaluation for ciprofloxacin. **a** PRED versus observed concentrations; **b** IPRED versus observed concentrations; **c** CWRES versus time; **d** CWRES versus PRED concentrations; **e** QQ-plot of the distribution of the NPDE versus the theoretical *N* (0, 1) distribution; **f** histogram of the distribution of the NPDE, with the density of the standard Gaussian distribution overlaid. *PRED* population predicted concentrations, *IPRED* individual predicted concentrations, *DV* observed concentrations, *CWRES* conditional weighted residuals, *NPDE* normalized prediction distribution errors, *SC* opportunistic blood samples

(e.g. 0.334, 0.330 and 0.366 L/h for the SC, TR, and full model, respectively, with 6 % relative standard errors of CL for all three models. The volumes of distribution at steady state ($V_{SS}$, sum of $V_1$ and $V_2$) were also quite similar, with typical estimated values of 4.15, 4.05 and 3.79 L for the SC, TR, and full model, respectively. However, the SC model had a different estimation of $V_1$ and $V_2$ compared with the TR and full models. For estimation of typical population pharmacokinetic parameters, the SC model showed similar results for CL and $V_{SS}$ estimation compared with the TR and full models, despite it yielding a different estimation of $V_1$ and $V_2$.

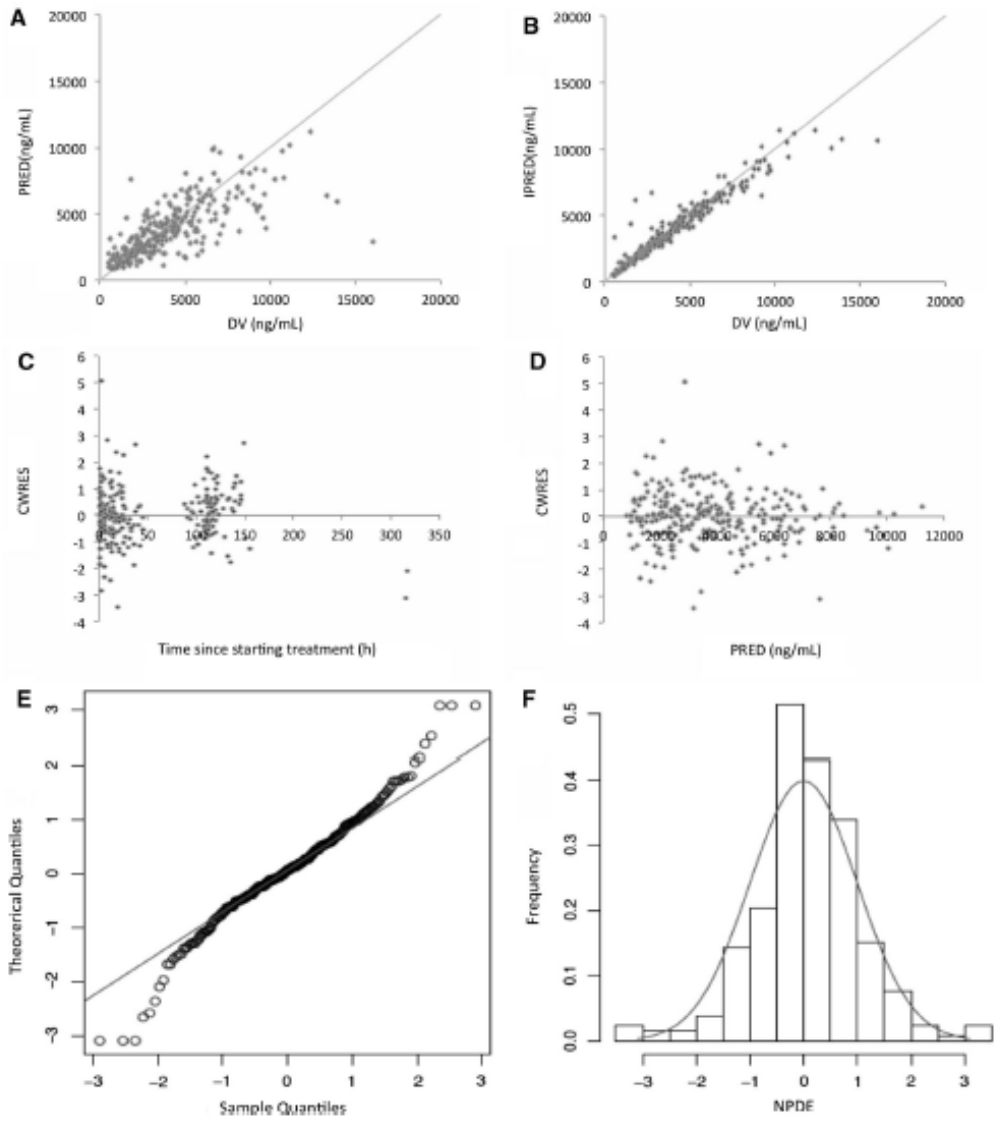


**Fig. 3** TR model evaluation for ciprofloxacin. **a** PRED versus observed concentrations; **b** IPRED versus observed concentrations; **c** CWRES versus time; **d** CWRES versus PRED concentrations; **e** QQ-plot of the distribution of the NPDE versus the theoretical *N* (0, 1) distribution; **f** histogram of the distribution of the NPDE, with the density of the standard Gaussian distribution overlaid. *PRED* population predicted concentrations, *IPRED* individual predicted concentrations, *DV* observed concentrations, *CWRES* conditional weighted residuals, *NPDE* normalized prediction distribution errors, *TR* predetermined (i.e. scheduled) samples

### 3.2.5 *Predictive Performance in an External Dataset*

The performance of the developed models was further evaluated in an independent group of 14 neonates and young infants, with a mean [standard deviation (SD)] gestational age of 33.9 (4.1) (range 26.0–39.0) weeks, a mean (SD) postnatal age of 32 (21.2) (range 2–84) days, a mean (SD) weight of 2701 (1352) (range 1020–5800) g and a mean (SD) serum creatinine concentration of 36.1 (25.5) (range 18.0–136.0) μmol/L. A total of 23 concentrations consisting of peak, trough and scavenged samples were available and ranged from 98 to 13,600 ng/mL. The individual predicted concentrations were highly correlated with measured concentrations for all three models ($r^2$ 0.96 for the full model, 0.95 for the TR model, and 0.97 for the SC model; $p < 0.01$ in all cases). The median PEs were

**Table 4** Population pharmacokinetic final model parameters of ciprofloxacin and bootstrap results

| Parameters | PK opportunistic sampling | | | Predetermined PK sampling | | | Full PK sampling | | |
|---|---|---|---|---|---|---|---|---|---|
| | Estimate (RSE %) | Bootstrap ($n = 500$) | | Estimate (RSE %) | Bootstrap ($n = 500$) | | Estimate (RSE %) | Bootstrap ($n = 500$) | |
| | | Median | 5th–95th CI | | Median | 5th–95th CI | | Median | 5th–95th CI |
| $V_1$ (L) | | | | | | | | | |
| $V_1 = \theta_1 \times (CW/1955)$ | | | | | | | | | |
| $\theta_1$ | 0.60 (21.9) | 0.64 | 0.23–2.78 | 2.50 (9.4) | 2.41 | 0.88–2.92 | 1.97 (17.7) | 1.82 | 0.78 to 2.59 |
| $V_2$ (L) | | | | | | | | | |
| $V_2 = \theta_2 \times (CW/1955)$ | | | | | | | | | |
| $\theta_2$ | 3.45 (8.7) | 3.34 | 1.75–3.94 | 1.65 (17.5) | 1.75 | 1.24–3.32 | 1.93 (21.9) | 1.97 | 1.38 to 3.02 |
| $Q$ (L/h) | | | | | | | | | |
| $Q = \theta_3 \times (CW/1955)^{0.75}$ | | | | | | | | | |
| $\theta_3$ | 3.99 (16.0) | 3.14 | 1.04–4.38 | 1.31 (17.9) | 1.35 | 0.80–5.65 | 2.50 (32.6) | 2.62 | 1.02 to 5.41 |
| CL (L/h) | | | | | | | | | |
| $CL = \theta_4 \times (CW/1955)^{0.75} \times F_{age} \times RF \times F_{inotrope}$ | | | | | | | | | |
| $\theta_4$ | 0.344 (5.6) | 0.341 | 0.311–0.374 | 0.330 (6.3) | 0.329 | 0.294–0.363 | 0.366 (6.0) | 0.365 | 0.323 to 0.407 |
| $F_{age} = (GA/27.9)^{\theta_5} \times (PNA/27)^{\theta_6}$ | | | | | | | | | |
| $\theta_5$ | 1.81 (16.2) | 1.84 | 1.32–2.25 | 1.98 (14.8) | 1.98 | 1.50–2.45 | 2.11 (11.9) | 2.09 | 1.60 to 2.57 |
| $\theta_6$ | 0.484 (12.9) | 0.49 | 0.382–0.599 | 0.41 (15.1) | 0.41 | 0.31–0.52 | 0.494 (10.8) | 0.492 | 0.386 to 0.606 |
| $RF = EXP[(CREA-42) \times \theta_7]$ | | | | | | | | | |
| $\theta_7$ | −0.006 (26.4) | −0.006 | −0.0009 to −0.003 | −0.009 (22.8) | −0.009 | −0.017 to −0.006 | −0.003 (46.0) | −0.003 | −0.008 to −0.001 |
| $F_{inotrope}$ | | | | | | | | | |
| $\theta_8$ | / | / | / | / | / | / | 0.708 (10.9) | 0.719 | 0.572 to 0.869 |
| Interindividual variability (%) | | | | | | | | | |
| $V_1$ | / | / | / | 41.1 (61.5) | 40.5 | 9.8–69.2 | 48.1 (63.6) | 49.6 | 26.2 to 77.2 |
| $V_2$ | 28.1 (66.8) | 27.4 | 1.0–87.2 | 71.0 (51.6) | 61.1 | 1.0–91.8 | 49.3 (68.3) | 51.2 | 15.8 to 76.9 |
| CL | 37.1 (27.7) | 36.4 | 27.6–45.2 | 35.8 (24.5) | 34.9 | 25.9–42.3 | 33.2 (19.9) | 31.3 | 25.3 to 37.4 |
| Interoccasion variability (%) | | | | | | | | | |
| CL | / | / | / | / | / | / | 16.4 (55.6) | 16.6 | 9.2 to 26.2 |
| Residual variability (%) | 18.2 (22.6) | 17.7 | 14.1–20.9 | 19.2 (29.5) | 19.2 | 14.5–23.9 | 19.3 (28.2) | 18.7 | 14.8 to 23.1 |

In our population, 1955 g, 27.9 weeks, 27 days and 42 µmol/L are the median current weight (day of the study), gestational age at birth, postnatal age and serum creatinine concentration values, respectively

*CL* clearance, *CREA* serum creatinine concentration in µmol/L, *CW* current weight in grams, *EXP* exponential function, $F_{age}$ impact of age, $F_{inotrope}$ scaling factor applied for patients coadministrated with inotrope, *GA* gestational age at birth in weeks, *PK* pharmacokinetic, *PNA* postnatal age in days, *Q* intercompartment clearance, *RF* renal function, *RSE* relative standard error, $V_1$ central volume of distribution, $V_2$ peripheral volume of distribution

similar between each of the models: 0.3 % (5th–95th percentile: −9.1 to 18.0 %) for the full model, 1.4 % (5th–95th percentile: −11.5 to 25.1 %) for the TR model, and 1.1 % (5th–95th percentile: −13.2 to 7.9 %) for the SC model, indicating a good and consistent predictive performance of developed models on new patients.

### 3.2.6 *Predictive Performance in Dose Prediction*

The target attainment rates as a function of dose and age groups for a standard EUCAST (European Committee on Antimicrobial Susceptibility Testing) MIC susceptibility breakpoint of 0.5 mg/L are shown in Fig. 4. The curves showed a high overlap between the three models. A theoretical dosing regimen of 7.5 mg/kg of ciprofloxacin administered every 12 h to neonates (PMA < 34 weeks) resulted in a prediction that approximately 90 % of patients would achieve an $AUC_{24}$/MIC ratio of 125 h using the full model, 88 % for patients using the SC model, and 92 % for patients using the TR model. The dose prediction of ciprofloxacin based on the $AUC_{24}$/MIC using the SC model produced a result similar to both the full and TR models.

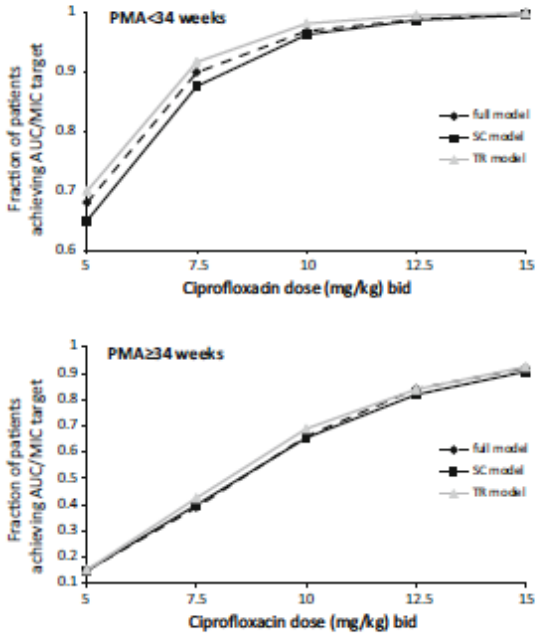


**Fig. 4** Target attainment rates in neonates and young infants. The target attainment rates for the 100 simulated trials for an MIC value of 0.5 mg/L is presented as a function of dose and sampling strategy. AUC/MIC target is 125 h. *MIC* minimum inhibitory concentration, *AUC* area under the concentration–time curve, *PMA* postmenstrual age, *bid* twice daily, *SC* opportunistic blood samples model, *TR* predetermined (i.e. scheduled) samples model

## 4 Discussion

Our data demonstrated that for ciprofloxacin in the neonate, an opportunistic sampling strategy enabled estimation of population pharmacokinetic parameters that were comparable with those from our prospective study [3]. They provide proof-of-concept of the utility of an opportunistic sampling paradigm to characterize the disposition of this, and, potentially, other antimicrobial agents, in neonates.

The issues in obtaining neonatal pharmacokinetic samples have been highlighted in regulatory guidelines. The EMA recommends that clinical trial-related blood loss should not exceed 3 % of the total blood volume during a period of 4 weeks and should not exceed 1 % at any single time, which means that a maximum of 2.7 mL/kg blood (assuming a total volume of blood of 90 mL/kg) could be obtained for investigational purposes [11, 12]. The US FDA recommends that volume and frequency of blood sampling can be minimized by using micro-volume drug assays and sparse sampling techniques, respectively [13]. Recently, the Medicines for Children Research Network (MRCN) also emphasized that it is preferable to obtain blood for research purposes during routine clinical care whenever possible [14]. Thus, both physiologic constraints and regulatory guidances suggest using fewer samples to support pharmacokinetic research objectives in the neonatal period.

The use of population-based pharmacokinetic approaches coupled with sampling paradigms that reduce the absolute number (and volume) of blood samples represents a preferred approach to support neonatal studies of drug disposition. Such an approach can be enriched through the use of residual blood samples (i.e. opportunistic samples) that are obtained as part of standard medical care. The use of opportunistic samples in this fashion can mitigate total blood loss (i.e. that used for clinical care and to support pharmacokinetic studies) and also reduce the risks associated with multiple blood sampling (e.g. number of times a vascular cannula needs to be accessed). Additionally, from an ethical perspective, the opportunistic sampling strategy may also facilitate parental consent for clinical trials. It has the potential to significantly lighten the burden of conducting necessary pharmacokinetic trial designs in neonates.

In the present study, ciprofloxacin was used as a model drug to evaluate the performance of an opportunistic sampling paradigm. According to regulatory guidelines [15–17], ciprofloxacin is a good example of a drug for which optimization of the dose for neonates may be best achieved using pharmacokinetic informed approaches, given that it has an established pharmacodynamic surrogate

**Table 5** Summary of published pharmacokinetic studies using an opportunistic sampling approach

| References | Drug | Age[a] | No. of patients | Total no. of samples (% SC samples) | No. of samples per patient[b] |
|---|---|---|---|---|---|
| Wade et al. [18] | Fluconazole | GA 26 (23–40)<br>PNA 2 (1–13) | 55 | 357 (39) | 6.5 (1–16) |
| Cohen-Wolkowiez et al. [1] | Metronidazole | GA 27 (22–32)<br>PNA 41 (0–97) | 32 | 116 (90) | 3.6 (1–15) |
| Cohen-Wolkowiez et al. [2] | Piperacillin | GA 25 (22–32)<br>PNA 17 (1–77) | 56 | 211 (96) | 3.7 (1–22) |
| Tremoulet et al. [19] | Ampicillin | GA 36 (24–41)<br>PNA 5 (0–25) | 73 | 142 | 2.1 |

*GA* gestational age in weeks, *PNA* postnatal age in days, *SC* opportunistic samples

[a] Median (range)

[b] Average (range)

endpoint dependent on attainable systemic drug exposure. Irrespective of the approach used, this clinical goal can only be achieved with confidence if the necessary pharmacokinetic parameter estimates obtained from any given approach for sampling are proven to be accurate.

The results from the current study demonstrate that the population pharmacokinetic model derived from an opportunistic sampling strategy provides a robust and accurate estimation of primary population pharmacokinetic parameters, and, in particular, CL and $V_{SS}$. Differences in the estimations of $V_1$ and $V_2$ compared with the TR and full models were most likely due to the timing of sampling episodes that supplied the scavenged samples. The timings of clinical sampling episodes are usually determined by the routine of the unit, and are generally clustered at the start of the working day. This leads to an overrepresentation of some sampling times and a reduced likelihood that samples were equally taken during the distribution phase for ciprofloxacin. Another issue is the variability in available sample numbers (e.g. 0–10 per patient) between patients whose samples were included in the SC model evaluation. These are common issues for our ciprofloxacin study., and for piperacillin and metronidazole pharmacokinetic studies [1–3]. The methodological aspect for designing an 'optimal' sampling-based opportunistic pharmacokinetic study still needs to be evaluated. Despite this limitation, the opportunistic sampling approach was able to identify the significant impact of covariates, such as gestational age, postnatal age, current weight and serum creatinine concentration, thus providing data which are important in the characterization of drug disposition in the neonatal population. Given the purpose of generalizing the appeal of an opportunistic sampling approach in population pharmacokinetic studies in neonates, we performed an external validation of three models in an independent group of patients. The results demonstrate similar predictive performance between each of the models, confirming the utility of an opportunistic sampling approach.

The generalizability of population pharmacokinetic studies using an opportunistic sampling approach depends on several key factors.

First, the density (both amount and time) of sampling. From a pharmacokinetic modelling technique prospective, if there are not enough samples and/or all samples are taken at only one fixed time (e.g. therapeutic drug monitoring [TDM] samples at trough concentration), this will impact the quality of the pharmacokinetic model. This seems to be a restricting factor in other populations but not in critically ill neonates. According to the patients' characteristics and clinical conditions, a large number of opportunistic samples are expected and many samples are taking during an intensive care setting to monitor vital signs in early life. In our experience, approximately four routine samples are currently drawn per day in critically ill neonates for different purposes, making the time of sampling variable. It overcomes the disadvantage of TDM samples with limited samples at a fixed time (i.e. trough and/or peak concentrations). The review of four previously published neonatal pharmacokinetic studies using opportunistic sampling confirmed that the density of sampling was not a problem to generalize the opportunistic sampling in neonates [1, 2, 18, 19]. The opportunistic samples accounted for 38–96 % of all the pharmacokinetic samples, and time of opportunistic samples covered the full pharmacokinetic profile (Table 5). Of note, as more routine samples will be scavenged in sicker neonates, the surrogate of severity of disease (e.g. use of inotrope) can be included in covariate analysis to evaluate its potential impact on pharmacokinetic parameters.

Second, the quality of sampling. The quality of sampling required to conduct an opportunistic pharmacokinetic study is the same as with 'classical' pharmacokinetic designs. In

order to ensure the quality of opportunistic sampling, a standard operation procedure to handle opportunistic samples, a case report form to record the precise sampling time of all opportunistic samples, and a drug administration history (including dosing and infusion time of each administration) should be used. This can be done only in a prospective study design. Close collaboration between physicians, clinical pharmacologists and research teams is indispensable to ensure the success of an opportunistic pharmacokinetic study.

Finally, the stability of the evaluated drug. The stability of the drug under evaluation may be a practical issue when performing an opportunistic sampling approach-based pharmacokinetic study. In two previous pharmacokinetic studies with an opportunistic sampling design, Cohen-Wolkowiez and colleagues reported that piperacillin concentrations of opportunistic samples were two- to tenfold lower than previously published values [2, 20, 21], and metronidazole concentrations of opportunistic samples were underestimated by approximately 30 % compared with the few timed specific pharmacokinetic samples. This observation is probably related to the long-term stability of evaluated drugs. Indeed, plasma was stored at −70 °C for a maximum of 32 months before analyses [1, 2], while long-term stability was evaluated only after 1 month of storage at −70 °C [22]. In the present study, ciprofloxacin remained stable (i.e. final concentration ≥90 % of initial concentration) for at least 1 year at −70 °C. As all ciprofloxacin samples were stored for a maximum of 8 months at −70 °C prior to analysis, there were no issues regarding frozen stability in the interpretation of the plasma concentration for a given sample.

## 5 Prospective and Conclusions

The challenges associated with performing pharmacokinetic studies in critically ill neonates can be mitigated using residual blood samples from specimens required for routine medical care (i.e. opportunistic specimens). As we have demonstrated for ciprofloxacin, an appropriately constructed population pharmacokinetic model using opportunistic samples can reliably estimate important pharmacokinetic parameters needed to support individualization of therapy. This is especially relevant for drugs where an exposure-based, pharmacodynamic surrogate (e.g. a target exposure) is available. In addition, the use of opportunistic samples to support pharmacokinetic studies in the neonate affords an economy of scale relative to improved study design through reduced total sample volume requirements, reduced risk associated with non-therapeutic blood sampling, and reduced parental concern associated with repeated blood sampling. The resources required to conduct an opportunistic pharmacokinetic study are the same as for other designs. The generalizability of a population pharmacokinetic study using an opportunistic sampling approach depends on the density and quality of sampling, as well as the stability of the drug under evaluation.

The present study is not intended to change the recommendation of using the sparse sampling design in neonatal pharmacokinetic study, but to provide an alternative approach (more adapted to neonatal clinical practice) to facilitate the pharmacokinetic study design in this vulnerable population. These two approaches complete each other. Indeed, mixing these two approaches may be an optimal way of maximizing the pharmacokinetic information of the drug under evaluation in neonates.

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**Conflicts of interest** Stéphanie Leroux, Mark A. Turner, Chantal Barin-Le Guellec, Helen Hill, Johannes N. van den Anker, Gregory L. Kearns, Evelyne Jacqz-Aigrain, and Wei Zhao declare no conflicts of interest relating to this work.

**Ethical approval** All procedures in this study were in accordance with the 1964 Helsinki declaration (and its amendments). The study was approved by the institutional ethics board and independent ethics board of the TINN project (EudraCT 2010-019955-23), and was also monitored by an independent safety monitoring board.

**Informed consent** Parental written consent to participate was obtained.

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## SECTION 1 – SPONSORSHIP AND RESEARCH GOVERNANCE RISK ASSESSMENT

| | | | |
|---|---|---|---|
| **Proposed Study:** Phase I, open-label pilot PK Study -TINN Treat Infections in Neonates Program | | | |
| **Proposed Co-Sponsors:** Liverpool Women's NHS Foundation Trust, Crown Street, Liverpool L8 7SS and University of Liverpool, The Foresight Building, Brownlow Street, Liverpool L69 3GL | | | |
| **Risk Assessment Conducted by:** | | | |
| **Date of Risk Assessment:** | | **Document Version:** | |
| **Risk/Hazard identified** | **Likelihood** Low Medium or High | **Impact** Low Medium or High | **Concerns and Recommendations for Mitigation & Management** |
| **Compliance with regulations** | **L** | **M** | The trial is of an investigational medicinal product. The sponsors (Trust) will need to assure compliance with the Clinical Trial Regulations 2004/1031 (as amended). |
| **Organisational Accountability** | **M** | **L** | **Contracts**: Co-sponsorship is confirmed by Liverpool Women's NHS Foundation Trust, Crown Street, Liverpool L8 7SS and University of Liverpool, The Foresight Building, Brownlow Street, Liverpool L69 3GL. A co-sponsorship agreement will be put in place between LWFT and University of Liverpool. An Agreement exists for the provision of Research Management Services between the University and Trust detailing the clinical research management of the study by the Trust (as the Co-ordinating Centre).<br>Clinical Trial Site Agreement will be prepared and signed at each recruiting site and the specialist analyst laboratory. No contract is required for the continuing care sites however a statement of responsibilities will be submitted through the Comprehensive Research Network service for Central NHS Permissions. Standard operating procedures will be prepared specifically for recruiting and non recruiting (follow up) sites to ensure the guidance is specific to their responsibilities. |

| | | | |
|---|---|---|---|
| | | | A Material Transfer Agreement will cover the transfer of samples to the laboratory for PK and DNA analysis. |
| Inadequate /poorly documented delegation to sites | L | M | Recruiting sites: Principal Investigators at recruiting sites will be take responsibility for the delegation of roles to the research team confirming each member is 'confident, competent, delegated and trained'. GCP certificates and curriculum vitae of team members will be held on the site trial file and the delegation log will be signed by both the PI and the team member specifying the roles they are delegated to do. Principal Investigators will meet with the Chief Investigator every 3 months to review recruitment and compliance with the protocol.<br><br>Follow Up Sites: a 'statement of responsibilities' and generic Site Specific Information form will be provided for follow up sites outlining governance requirements and the role of the PI in accordance with the Research Governance Framework (2005), transfer of Site-Specific Assessment to NHS R&D, and Centralised NHS Permissions CSP National Guidelines. |
| Poor quality control and quality assurance | M | M | The PI and CLRN nurse will be GCP trained and familiar with the protocol thereby able to ensure SAEs and SUSARs are reported within the time line stated in the protocol. Also, knowledgeable of the standard operating procedures specific to follow up sites. The recruiting sites will liaise closely when a baby is transferred to other hospitals to ensure the site team are given guidance on follow up requirements. |
| Inadequate monitoring & auditing | M | M | Monitoring by the Sponsor (Trust) will be undertaken according to a monitoring plan based on the outcome of the bespoke risk assessment. It is assumed that on site monitoring will be required. This will include GCP, Research Governance, source data checks, laboratory handling of samples and data reliability.<br>Data reliability is the greatest risk of this study and it will be important to review the 1st 10 Pharmacokinetic samples for quality control to ensure data is within the expected range. There is a risk that the data is not precise and that the audit tools are not sensitive to detect errors therefore trial tools will be developed to provide more than one source of data verification. |
| Poor archiving of study related information | L | L | Patient data will be managed in accordance with GCP, the Caldecott Guardian /National Information Governance Board and the Data Protection Act. Following the study the medical notes are archived using the NHS hospital appointed archiving services. The medical notes will be labelled as requiring archiving for 15 years (protocol requirement). The Sponsor (Trust) will monitor the storage of data is compliant with these regulations. TMF will include essential documents and version control ICH GCP section 8 [1] |

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| | | | |
| Inadequate patient safety monitoring | L | L | The Independent Data Safety Monitoring Committee will need to be put in place. The Chief Investigator will need to provide a summary of adverse events and the anticipated incidence for this patient population. |
| Study Design: , inadequate study powered recruitment | L | L | The TINN Consortium of Neonatal experts across Europe have contributed to the design of this study (www.tinn-project.org). Trial Adoption will be requested from Medicines for Children Research Network providing independent peer review of the study design and methods. A European Ethics Advisory Group is set up to ensure the study is designed to meet the requirements of this vulnerable patient group.<br>Feasibility will be undertaken to identify the number of babies for recruitment but if the target is not met further sites will be opened. Due to high mortality in this group babies will be over recruited to ensure the minimum target is met for Day 1 and Day 5 samples. |
| Inadequate costing of the study | M | M | The funding for the study includes a contingency allowance.<br>A monthly report will be provided to the Investigator and reviewed by the Research Support Office a quarterly basis Research Support Office |
| Insurance/Indemnity | L | L | The NHS Sponsor's indemnity is provided by the NHS Litigation Authority<br>As Co-sponsor the University of Liverpool (there is no exclusion for neonates or children in the University insurance policy) |

## Approval

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Chief Investigator: | | Date: | | Sponsor: | | Date: | |
| | | | | Sponsor: | | Date: | |

# SECTION 2 – IMP RISK ASSESSMENT AND SAFETY MONITORING

## Risk Assessment of the Investigational Medicinal Product

(Drug risk assessment based on SmPC, BNF Children, protocol background papers, data on other quinolones, British National Formulary for Children and the MHRA Public Assessment Report )

**Study Title:** LW0852: To evaluate the pharmacokinetics, tolerability and short-term safety of ciprofloxacin in neonates with suspected (or proven) Gram Negative infection. Phase I, open-label pilot PK Study -TINN Treat Infections in Neonates Program

Phase I, open-label pilot PK Study -TINN Treat Infections in Neonates Program

**EudraCT:** 2010-019955-23

**Co-Sponsors:** Liverpool Women's NHS Foundation Trust, Crown Street, Liverpool L8 7SS and University of Liverpool, The Foresight Building, Brownlow Street, Liverpool L69 3GL

| **Risk Assessment Conducted by:** | **Date of Risk Assessment:** | **Document Version:** |
|---|---|---|

| | |
|---|---|
| **Risks associated with trial IMP/interventions**<br>☑ **Type A** ≡ Comparable to the risk of standard medical care<br>☐ **Type B** ≡ Somewhat higher than the risk of standard medical care<br>☐ **Type C** ≡ Markedly higher than the risk of standard medical care | Ciprofloxacin is prescribed by the clinical team as part of standard medical care not for the purpose of research. This is a Pharmacokinetic clinical trial that requires additional monitoring and collection of safety data therefore the risks from the study interventions are associated with monitoring but additional safety aspects of the IMP will be monitored in detail. Patients will be intensively monitored as per standard of care as they are critically ill.<br>**Ciprofloxacin Guidelines:** The SmPC dated 2/1011 includes the use of this drug indicated for severe infection in children. Ciprofloxacin (intravenous) is licensed for use in children over 1 year of age in the British National Formulary for Children 2011, the neonatal use is off label, but the neonatal dose is included in the British National Formulary for Children. The Liverpool Women's Neonatal Late onset Sepsis policy includes the use of Ciprofloxacin.<br>**Published Evidence:** A systematic review of adverse effects in paediatrics identified 105 articles involving 16 184 paediatric patients (they found that the musculoskeletal adverse events were reversible) [2]. A prospective study of adverse events found that treatment of neonatal sepsis with ciprofloxacin resulted in no short term hematologic, renal or hepatic adverse effects and did not appear to be associated with clinical arthropathy or growth impairment at 1 year follow up evaluation [3]. A Systematic review specific to neonates found 32 cohort studies or case reports[4] .<br>**Established Practice:** Survey: 26% (50 /193) neonatal units use Ciprofloxacin at least occasionally in Europe (http://www.tinn-project.org/).<br>Ciprofloxacin has been administered off label to neonates for over 20 years as part of clinical care and published evidence [3-8].<br>**Off label use** is established practice for children and neonates, 55% drugs are administered off label to neonates [9].<br>This study will contribute to a non-commercial Paediatric Investigational Plan to contribute to licensing this drug specifically for neonates and influencing prescribing guidelines for neonates internationally. A study to evaluate the pharmacodynamic microbiological and clinical outcome of babies administered a lower dose 5mg/kg 12hourly is being evaluated by the research team at present.<br><br>An Investigator Brochure is replaced by the SmPC. As the IMP is used off label, the trial will require authorisation by MHRA, rather than notification to MHRA. |

| IMP/ Intervention | Body System | Hazard | Likelihood Rare, Low Medium or High | Mitigation | Comments |
|---|---|---|---|---|---|
| Ciprofloxacin administered Intravenously | Joints | Arthropathy/ tendinopathy | M | Assess mobility pain redness prior to IMP administration and on set days during infusion and follow up for 6 weeks<br>Nurses /parents are asked to report any signs of tenderness in joints when handling the baby | Neonates are not weight bearing so it is difficult to assess arthropathy. The patients may be recruited to a separate MRI study to assess joints. Pre clinical studies to assess joints in mice.<br>Further examination will be undertaken by the Consultant Neonatologist if tenderness is reported. |
| | Vein | Phlebitis | L | Validated Visual Infusion Phlebitis Score monitored by the nurse during after infusions for 6 hours | Recorded in CRF |
| | Liver Function | Failure /pancreatitis | L * | Base line bloods and day 1-7 and day 10 and >daily clinical assessments are part of intensive care management | Daily clinical blood results are required for clinical care used for monitoring to minimise blood sampling.<br>Reference ranges for neonates stated in the protocol.<br>Values out with the normal range are associated with critical illness therefore may not be related to the intervention. |
| | Renal Function | Failure<br>Crystalluria | L * | | |
| | Blood cells | Deranged | L* | | |
| | Gastro-intestinal | Colitis if severe and persistent consider Clostridium Difficile | M | Nappies are routinely weighed to estimate urine output and faeces when stools are watery.<br>Stool samples sent to microbiology if colitis suspected<br>Weekly surveillance of rectal swabs by bacteriology. | |
| | Anaphylaxis /Allergy | | R | Intensive or High Dependency Level of Monitoring<br>Daily + Clinical Assessments<br>Vital signs monitored frequently /continuously | Signs or allergic reaction, rash/photosensitivity/ cardiac arrhythmias /convulsions and other conditions are systematically reported by nursing staff in the electronic patient data system and can be monitored daily as required by the researcher. |
| | Skin: | Rash/photosensitivity | M | | |
| | Cardiac | ventricular arrhythmia, QT interval prolongation | R | | |
| | Neurological | Convulsions | M | | |
| | Syndromes | Stevens Johnson /Lyell | R | | |

## Pharmacovigilance and processes that have been put in place to mitigate risks to participant safety (IDMC, independent data review,...)

Ciprofloxacin is considered a low risk as the drug is given for clinical care, the decision to prescribe is independent of the decision to enrol a baby into the trial. The study population are a vulnerable group who are critically ill therefore many adverse events are anticipated due to the nature of critical illness triggered by 1) clinical condition 2) clinical interventions 3) other drugs 'poly pharmacy' therefore causality is difficult to determine. There is an anticipated high mortality of 44% based on retrospective data of babies with Gram negative Sepsis over the last 6 years at this site.

Due to the anticipated high number of Adverse events (AE) and Serious Adverse Events (SAE) for reasons outlined above and the logistics of the trial, the protocol will outline which events need to be recorded by the investigator onto the CRF and which need to be reported immediately to the Sponsor (as per Regulation 32 (4)[10], ICH GCP 4.11.1 [1] and CT3 5.1.9 [11]) . The investigator is required to record all SAEs in the CRF and those AEs identified during the risk assessment as requiring recording. The investigator must report immediately to the Sponsor all SAEs, except those that are identified in the protocol as 'anticipated' events.

The process for reporting anticipated Serious Adverse Events (whether related to the IMP or not) to the Sponsor (Trust) will be defined in the protocol and standard operating procedure. The Sponsor (Trust) will monitor the incidence of anticipated SAE, if this increases during the trial they are required to report this as a SUSAR to the MHRA and NRES. All SAE whether related to the IMP or not will be recorded in the case report form and a summary provided for the Sponsor (Trust) and DMC.

The protocol will list the anticipated Serious Adverse Events (SAE) commonly seen in extremely premature babies less than 28 week and 34 weeks based on the incidence at Liverpool Women's NHS FT between 1980 and 2004.

| **SAE anticipated in critically ill /premature neonates** | | |
|---|---|---|
| **SAE** | **Estimated Incidence < 28 weeks gestation** | **Estimated Incidence 28 - 34 weeks gestation** |
| Death | 20% | 8% |
| Necrotising Enterocolitis | 15% | 3% |
| Intracranial abnormality | 15% | 6% |
| Supplementary Oxygen | 55% | 6% |
| Patent Ductus Arteriosus | 25% | 8% |
| Retinal Surgery | 5% | 0.14 |
| Pulmonary Haemorrhage | 5% | 0.5% |

**Serious Adverse Events that are not 'anticipated'** or **Suspected Unexpected Serious Adverse Reaction** (SUSAR) will be reported to the sponsor (Trust) within 24 hours by the Principal Investigator (LWH R&D Office). SUSARs require expedited reporting to the MHRA and REC within 7 or 15 days in accordance with regulation 33 [10].

**Adverse Events** (non-serious) that are identified by the Sponsor as requiring recording in the CRF following a risk assessment of the IMP will be recorded in the CRF (SmPC for all quinolones, protocol back ground papers, British National Formulary for Children and the MHRA Public Assessment Report). These include

arthropathy /phlebitis / altered liver or renal function/pancreatitis/deranged blood cells, gastro intestinal, skin, cardiac and fitting. The case report form and trial monitoring tools are designed to collected these events systematically; they only require immediate reporting to the Sponsor (Trust) if they are assessed by the Principal Investigator as Serious (regulation 32 (5) [10]).

**Adverse events (non-serious) that have been identified in the risk assessment as uncommon** but may be relevant are identified in the standard operating procedure and should also be included in adverse event summary reports/case report forms including: Gastro intestinal, anaphylaxis /allergy /skin: rash/photosensitivity /cardiac arrhythmias: ventricular arrhythmia, QT interval prolongation Syndromes: Stevens Johnson /Lyell /Neurological: convulsions. As these are not critical to the evaluation of the safety of the trial (regulation 32 (5) [10]) they are only recorded in case report forms and included in DMC reports unless they are serious when they are reported as above.

**All other adverse events** in this patient population that are common during critical illness including for example altered desaturations (oxygen levels), diarrhoea, vomiting and tachycardia will not be recorded as the Sponsor (Trust) does not feel these are critical to the evaluation of safety (regulation 32 (5) [10]), unless the attending clinician assesses the event as serious or has a temporal relationship to the administration of Ciprofloxacin

**Concomitant Medications** - Critically ill patients are likely to be administered many medicines simultaneously, which make an assessment of relatedness to Ciprofloxacin difficult. All concomitant medications will be recorded in the CRF. Drugs that are known to interact have been identified in the SmPC; these include drugs metabolised by CYPIA2 that can increase serum concentration of drugs including Theophylline. Other drugs administered to neonates that can alter levels include Caffeine, Phenytoin and oral anticoagulants (including Warfarin). Concurrent administration with NSAID can provoke convulsions. These groups of drugs and others relevant to covariate analysis including antimicrobials, analgesia and inotropes will be recorded within the CRF.

**Monitoring Period:** monitoring of adverse events will commence on the first day the baby is eligible and when Ciprofloxacin has been administered and for the following 42 days (the last study procedure). Section 3.0 [12]

**Summary Report of Adverse Events:** The Investigator will assess all AEs and SAEs on monthly basis. A summary of SAE (including anticipated SAE) will be sent to the Sponsor (Trust) every 3 months. The Data Monitoring Committee will assess SAEs on a 6 monthly basis. Any potential increase in severity or incidence that is detected will then require a report to be made to the sponsor. Annual report will be made to NRES/MHRA/Data Monitoring Committee in accordance with Regulation 35 [8].

**The protocol will specify the safety monitoring procedures as described above and there will also be a trial specific SOP.**

## Approval

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Chief Investigator:** | | **Date:** | | **Sponsor:** | | **Date:** | |
| | | | | **Sponsor:** | | **Date:** | |

# SECTION 3 – BESPOKE TRIAL RISK ASSESSMENT

| Bespoke Risk Assessment (participant safety relating to the IMP, study design, methods, safety and rights and reliability of results) | |
|---|---|
| **Study Title:** LW0852: To evaluate the pharmacokinetics, tolerability and short-term safety of ciprofloxacin in neonates with suspected (or proven) Gram Negative infection. Phase I, open-label pilot PK Study -TINN Treat Infections in Neonates Program<br>**Phase I, open-label pilot PK Study -TINN Treat Infections in Neonates Program** | |
| **EudraCT:** 2010-019955-23 | |
| **Co-Sponsors:** Liverpool Women's NHS Foundation Trust, Crown Street, Liverpool L8 7SS and University of Liverpool, The Foresight Building, Brownlow Street, Liverpool L69 3GL | |
| **Risk Assessment Conducted by:** | |
| **Date of Risk Assessment:** | **Document Version:** |

## 1. IMP

| | Risk identified | Likelihood Low Medium High | Concerns | Mitigation or Adaptation | Monitoring methods to address |
|---|---|---|---|---|---|
| Drug is administered off label (licensed for children over 1 year) | Drug dose | M | Yes. Dose is insufficient to treat the infection or toxic to the baby | • The dose is estimated on paediatric licensed doses for children over 1 year of age<br>• Previous experience of use in neonates(see section 2)<br>• Interim data will be analysed by comparing PK/PD data to determine whether a change in dose is required (after 1st 10 babies)<br>• The TINN consortium of neonatal experts will evaluate interim data (based on optimal dosing model from adult studies).<br>• Intensive care babies have continuous or regular clinical monitoring and daily assessments of vital signs, biochemistry and haematology.<br>• DMC will review the 1st 10 babies adverse events and at 6 monthly intervals.<br>• Case report form will systematically collect data on vital signs during the 1st 10 days of administration. | • The Sponsor (Trust) /Monitor will ensure an interim analysis takes place as planned.<br>• Monitor to confirm that a charter is in place for TINN consortium and the DMC to outline their duties and functions in the trial conduct.<br>• |

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| Storage | Hospital stock of IMP may be stored in up to 30 wards between both sites. Impractical to monitor temperature. | L | None. | • Stability Data from pharmaceutical company for safe storage up to 40oC<br>• The standard hospital pharmacy practice does not require the temperature of drugs to be monitored outside of pharmacy in ward stock cupboards (Duthie Report - the safe and secure handling of medicines issued by the Royal Pharmaceutical Society 2005)<br>• Store as per standard hospital practice exempt from temperature monitoring. | • No checks required on storage areas by on site monitoring. |
| Drug Labelling | Impractical to label. Used as normal clinical practice. No risk. | L | None. | • Annex 13 labelling is not required as the drug is supplied by the NHS hospital as standard care. Regulation 46 applies. This is covered in protocol/CTA application. | • None. |
| Drug Accountability | Records must be maintained for the precision of the dose and exact time given otherwise PK results will be invalidated. | H | Yes. Impact on reliability of results from PK analysis. | • Hospital pharmacy will dispense the IMP.<br>• There are no requirements to record the batch number or expiry date or site level accountability records.<br>• Patient level accountability records are essential, precise documentation for recording infusions, dosage and exact time the drug is administered.<br>• Training of clinical staff (200+- per unit) will be undertaken and recorded.<br>• Generic drug - the same product , Claris, used at both recruiting sites<br>• No requirement for full accountability records, as the drug is prescribed as part of clinical care. | • Monitor to ensure completion of patient infusion records. SDV of this data recommended.<br>• Training records review (could be centrally done) |
| Infusion Practice – exact time the drug reaches the blood is required | Small volumes in neonates mean the drug rate is low and may not reach the blood for a long period (up to 24 minutes) | M | Yes. Incorrect time recorded for dosing – impact on PK analysis. | • Standard operating procedures are prepared specific to the recruiting site's drug infusion practice – the infusion line will be primed with ciprofloxacin to prevent delays in the drug reaching the blood.<br>• Record which IV line the drug is infused (particularly when a long line or central Broviac lines are in place with large dead space)<br>• Calculate the volume in the line between the patient and the drug prior to infusion and the rate of the infusion to correct the time the drug reaches the blood. | |
| | Lines not | M | | | |

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| | flushed after the infusion within the 3 minutes sampling period | | | • If there is a deviation from the planned time due to other clinical priorities details will be recorded on the sample schedule.<br>• Trial Training all 200 clinical nurses for each site (consistent with GCP for that task) | |
| | Dead space in infusion lines /broviac lines can be >2mls which takes 20 minutes to reach the blood if infused at 6ml/hour | M | | | |

| 2. Patient Safety | | | | | |
|---|---|---|---|---|---|
| **Area** | **Particular risk identified** | **Likelihood Low Medium High** | **Concerns** | **Mitigation or Adaptation** | **Monitoring methods to address** |
| Blood sampling<br><br>3 samples on day 1<br>3 samples on day 5<br><br>Scavenged when a biochemistry samples is collected for clinical care | Skin trauma | L | Yes. Heels already pricked /bruised | • Skilled staff trained in sampling neonates for clinical care<br>• Use of neonatal lancet<br>• Trust training policy for blood sampling | • Sample of Training records review (could be centrally done) |
| | Pain | L | Yes. Distress to parent and baby | • Use arterial or central line when possible<br>• Sample taken when clinical samples are required when possible from one heel prick planned with other cares when possible<br>• Sample planner agreed with the clinical team to ensure samples are collected at the same time as clinical bloods when possible by selecting the sample schedule in line with clinical blood times. | • Monitor to review sample planner and sample times to confirm baby is not having unnecessary sampling times.<br>• To monitor a selection of patients (not 100%). |
| | Blood loss | L | Yes. Increased risk of transfusions (For a neonate 500g weight the total blood volume | • Minimum volume 0.2 ml and sampling episodes reduce from 3 to 2 samples per day samples for babies less than 1000grams weight<br>• Limit participation to one study that requires blood samples or within the volume allowed by the MCRN EMA guidance | • Monitor to confirm not participating in another trial involving blood draw as part of eligibility check. |

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| | | | is only 40mls +-) | for neonatal sampling in clinical trials [13, 14]. For research - 3% total blood volume during 4 weeks or 1% at any single time based on a total volume of blood 80- 90 ml/kg body weight. | |
| Lumbar Puncture for CSF sample. | CSF sample – lumbar puncture<br>Pain<br>Loss of CSF volume<br><br>Precision for the time the sample is collected | L | Yes.<br><br>Disturbing the baby<br>Dehydration<br><br>PK analysis of CSF | • CSF samples will only be collected if the procedure is required clinically –additional CSF taken during the same procedure<br>• 0.2ml will be collected after the clinically required samples are taken.<br>• Trial label and sample bottle left on the cot with instructions for the person performing the procedure to add the time collected and store in the freezer -20oC | Freezer temperature monitored daily |
| DNA Sample | Mouth swab /blood volume | L | Yes.<br><br>Disturbs the baby<br><br>Dislodging endotracheal tube | • Buccal sample taken with other cares. Minimal handling is required to allow neonates maximum rest for growth and development.<br>• Check with the clinical team if an endotracheal tube is in place that it is secure –samples taken by intensive care trained staff for ventilated babies.<br>• Blood scavenged from EDTA samples | |
| Faeces | Required week 4 -6, possibly after discharge | L | Yes. Baby may be at another hospital or at home | • R&D approvals for 40 other hospitals and PI at each follow up site to allow samples to be collected<br>• Parents to send from home in Category B packaging provided by the trial staff conforming to the Health and Safety Executive Guidance[15]. | Collation and checks of approvals (central monitoring). Maintenance of approved site list. Monitoring to check follow up at approved sites (can be done centrally). |

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| Drug adverse effect or reaction | Complexity of pharmacovigilance in critically ill babies | L | The IMP is used off label and risks associated with the drug relate to data from adult studies.<br><br>Due to the complexity of adverse effects or reactions in critically ill babies there is a risk of not being able to identify events related to the IMP. | • A safety monitoring plan is in place and a DMC is established with charter/procedures. All adverse event reported to the sponsor (Trust) as per protocol are summarised for DMC at 6 monthly intervals to ascertain whether the risk of expected SAE has increased above the anticipated incidence (see Section 2).<br>• Pharmacovigilance procedures are based on a risk assessment of the IMP and anticipated adverse events in critically ill babies.<br>• SAE/SAR/AE/AR record and reported as they occur by the Principal Investigator as per protocol. SAE/SAR reports are sent to the Sponsor (Trust) and checked by an independent clinician (to assess expectedness for SAR).<br>• The protocol includes reference safety information (RSI) for the expectedness of suspected adverse reactions (serious and non-serious) to allow comparison with the actual incidence –reviewed 6monthly by the DMC. The RSI will be as per table section 2 Pharmacovigilance<br>• Routine clinical monitoring is continuous or at frequent intervals in intensive care patients to detect potential reactions<br>• Concomitant medications are recorded in the CRF for the period the IMP is administered | • Monitor to sample Source verification Data for AEs being reported as per protocol<br>• Monitor to check DMC undertaking regular safety reviews. |
| Eligibility | Ineligible patients recruited | L | Patients are eligible if prescribed ciprofloxacin and less than 52 weeks post menstrual age.<br><br>They are excluded if under 5 days age post birth or not likely to survive in the judgement of | • Prior to any study procedure a doctor looking after the baby will determine whether the baby meets the eligibility criteria and that they have made a clinical decision to allow the baby to participate in the trial.<br>• A form 'Determine Eligibility' includes the eligibility and exclusion criteria is completed by the doctor looking after the baby on the day the study starts and filed in the case notes.<br>• Staff training tools and a log of training.<br>• Recruitment checklist (version controlled and consistent with protocol). | The Monitor will confirm a medical review of criteria has been completed by checking the 'determining eligibility form'.<br><br>The eligibility will also be confirmed against the electronic patient data system for 30% of babies recruited. |

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| | | | an attending physician. | | |
| | Recruitment into more than one study allowed in the eligibility criteria | L | Yes. Risk to PK analysis if other medications administered as part of other trials not recorded. | • Review other trials taking place at recruiting sites to assess whether they present any issues for this trial.<br>• (see drug interactions section2) | |

| 3. Patient Consent, Rights and Confidentiality | | | | | |
|---|---|---|---|---|---|
| **Area** | **Particular risk identified** | **Likelihood Low Medium High** | **Concerns** | **Mitigation or Adaptation** | **Monitoring methods to address** |
| Consent | Yes. Vulnerable subjects: premature baby. Multiple trials in place at the same time | M | Yes. Parents concerned about allowing further procedures to their baby when their baby is critically ill. Stress due to requests for many trials. | • Information sheet includes 20 elements of consent ICH GCP (4.8.10) [1]. The consent form asks parents to sign to say they have read the information sheet.<br>• Consent processes and documents are approved by the REC and Patient Public Involvement representatives.<br>• Ethical issues are monitored by a European Ethics Advisory board in addition to NRES to ensure the protocol is ethically approved across Europe<br>• Staff trained in paediatrics /neonates will describe the procedures and discuss specific concerns regarding sampling [16]<br>• Consent processes are described in the protocol - prospective consent is preferred but deferred 'emergency consent' may be obtained according to regulation 2006 No 2984 [17] with approval by NRES.<br>• Researchers will co-ordinate their approach to parents and advise parents how many trials they are likely to be approached about during the admission.<br>• Researchers will be trained in paediatric consent/GCP/Trial protocol and law on parental responsibility (Children's Act 1989 /Adoption and Children Act 2002 www.opsi.gov.uk/acts/acts2002/kpga)<br>• The Principal Investigator and the research doctor/nurse will | • Check of records to ensure consent was given by someone with parental responsibility.<br>• Record time/date consent taken prior to procedures or record the use of deferred consent<br>• Check of 30% consent forms<br>• Check delegation log<br>• Check training record for the research team<br>(above could be achieved without a site visit) |
| | Parental responsibility | L | Yes. Failure to obtain consent from someone with parental responsibility. | | |
| | Parents not informed adequately about the study | L | Yes. Parent/Guardian stress due to critically ill baby impacts on capacity to consent - unable to concentrate on the informed | | |

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| | | | consent process | jointly determine that they are 'confident competent and delegated' to undertake consent.<br>• The PI will sign the delegation log for each site.<br>• Prospective consent allows parents more time to consider the information fully.<br>• Clinical team trained in the study able to answer parents questions<br>• Assess parental capacity to consent at the time by asking parents open questions<br>• Provide information to both partners when possible.<br>• Documented check on parental responsibility (if unmarried fathers sign the consent form they will be asked to add that they are named on the birth certificate.<br>• Explain the study is voluntary participation<br>• Telephone consent may be requested if parents are not available but researchers will attempt to provide them with the information first. Clinical team member will phone parents then witness the researcher consenting.<br>• Parents may opt to consent separately for 1) drug levels, 2) CSF and 3) DNA by signing separately for each component on the same form. | |
| | Coercion. Parents feeling obliged to participate due to the care given by the clinical team. | L | No. | | |
| | The study drug can be prescribed at any time of the day or night when parent may not be available – sampling required within 3 minutes | M | Yes. Parents not present at the time consent is required. Failure to obtain consent or loss of recruitment of subject. | | |

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| Information leaving the hospital & Data Protection | Medical data required by external teams in UK and Europe<br><br>Samples are labelled and sent to other laboratories and hospitals with documents. | L | Yes. Breach of subject confidentiality. | • Data will be anonymised before sending to any external hospital or laboratory.<br>• Data protection process are approved by NRES, Trust R&D and consistent with NIGB guidance.<br>• Parents are aware that we will contact their GP<br>• Clinical data is anonymised when used outside the clinical environment.<br>• The information sheet describes to parents how data is anonymised<br>• Data stored on a clinical database- this electronic CRF does not contain any patient identifiers and is password protected.<br>• DNA samples are anonymised prior to sending them to the genetics laboratory<br>• DNA extraction methods prevent information relating to other clinical conditions being identified prior to being anonymised.<br>• Process in place at labs to raise alert and implement corrective actions if labels contain patient identification data.<br>• Laboratory staff are GCP trained and employed by the NHS Trust.<br>• Medical notes are labelled to be archived by the Trust archiving services for 15 years<br>• Trial data is stored in a lockable cabinet in a lockable room GCP compliant | Check all sample labelling procedures and sample labels to confirm no fields for patient identifiers (centrally) |

## 4. Reliability of Trial Results

| Area | Particular risk identified | Low Medium High | Concerns | Mitigation or Adaptation | Monitoring methods to address |
|---|---|---|---|---|---|
| CRF data | Reliability of recording data<br><br>Covariates: weight, postmenstrual age, postnatal age and serum creatinine needed for PK analysis. | M | Inaccurate data. Electronic CRF not fit for purpose. Yes. Inaccurate covariate data – incorrect PK analysis. Weight varies daily/weekly in babies due to growth, altered nutrition and fluid shift or renal failure. | • The electronic CRF Designed and validated. The electronic CRF is approved by the Trial Team. System has password access, audit trail and back up.<br>• Hard copy of the CRF is completed in the clinical area then entered onto the ECRF.<br>• Hard copies are stored as source data stored at the recruiting sites | The monitor will check the E CRF against source data and the paper CRF for 30% of recruits.<br><br>A written statement from the ECRF company regarding GCP compliance will be filed on the TMF |
| | | L | | • PK Model developed by expert neonatal PK team within the TINN consortium<br>• Weight recorded at birth, start of study, the end of PK samples and on discharge. | Source data verification of covariates required as important for trial results. |
| Con Meds | Poly pharmacy | L | Yes. Up to 20 medicines administered concomitantly to each baby. There is a risk of metabolism interactions - potential to impact on PK analysis. | • All concomitant medications recorded during the administration of the IMP are recorded<br>• Specific drugs administered to neonates identified as having potential interactions for quinolones by the SmPC and Public Assessment Report (MHRA) are identified in section 2<br>• Caffeine and phenytoin are drugs with known interactions and are commonly prescribed therefore we are recording further details regarding the dose and time of administration.<br>• Analgesia may mask arthralgia therefore will be recorded in the CRF<br>• Analysed as covariates | Source data verification of concomitant medications – (sample to assure reliability of CRF data.) |
| Group Allocation at Recruitment | To achieve the minimum 5 babies per age group – distributed over Groups A and C or B and D | L | None. Potential risk of allocation errors result in imbalanced groups. | • The sampling group is allocated by the researcher following discussion with the clinical team regarding the best time for sampling the baby to coincide with the clinically required samples (to minimise disturbing the baby). Also based on whether the dose is prescribed 8 or 12 hourly.<br>• Record the number of recruits allocated to each sub group aiming to balance the groups throughout the study.<br>• The study is not comparing outcomes of one group with the other, groups are chosen to provide representation of drug | Summary of recruitment allocation maintained throughout the study. |

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| | | | | levels at different time points. | |
| Blood Sampling | Precise timing of samples necessary<br><br>Administering the drug infusion and taking samples sampling requires high level of precision and accurate timing | H | Yes. Samples collected out with the tight time frame of 3 or 10 minutes – PK analysis invalid.<br><br>Yes. Training large clinical teams – ensuring all those involved have appropriate training to ensure timings are recorded correctly. | • Advice to avoid sampling during an infusion<br>• Cot side guide for 5 main point of the study<br>• Cot side recording charts<br>• Record of times can be cross checked on multiple sources (blood gas analyser/phlebotomist record nursing notes/ arrival time in the laboratory recorded).<br>• Training for laboratory staff, phlebotomist and all clinical staff 200 at each intensive care unit<br>• Hand holding by researchers and research network staff to ensure a named clinical nurse /doctor is responsible and updated on each shift covering each patient.<br>• Label designed for the study are completed with the time the sample was taken<br>• Detailed SOPs and monitoring tools to ensure precision is achieved, monitored, recorded CRF and cross checked for quality control<br>• Time sample collected written on the form and a back up log<br>• Clocks checked in clinical areas/season changes<br>• Standard operating procedure for scavenging samples<br>• Scavenged from clinically required biochemistry samples<br>• Phlebotomists are trained in the study and add a label to the scavenged sample when they take the blood and hand write the time on the label and keep as record on site.<br>• The laboratory record the study ID number / time /date on the bottle on the label with the patient study ID (scavenged samples) | • Monitor to check records and undertake SDV to confirm sampling time accurate and procedures followed. Onsite visit needed.<br>• .PK analysts adjust the modelling based on the data but the team will aim to balance recruitment among groups |
| | Scavenged Samples Precise Timing | H | | | |

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| | Storage/preparation /transfer | L | Yes. Invalid assay results from inappropriately stored samples. | • Standard operating procedures detail the storage and handling of samples<br>• Blood samples are stored at -20oC CSF samples stored at -80oC. Laboratory freezers are alarmed to the central switchboard system and temperatures recorded daily.<br>• Hospital approved courier transported within Category B standards and for transport on dry ice [15]<br>• The courier agrees to deliver by 12 noon the following day and sufficient dry ice is provided for 3 days in case of delay. In the event no dry ice present on arrival then the samples are not included.<br>• The Laboratory is certified by Clinical Pathology Accreditation Standards for Medical Laboratories ISO 15189. and authorised to store and process samples<br>• Clinical samples are centrifuged for clinical analysis and any remaining plasma is scavenged for additional data when the time has been recorded on the clinical sample with a study label – nurses and phlebotomists are trained to provide this information.<br>• Laboratory staff GCP and trial trained.<br>• Controls in place for shipment, receipt & chain of custody records | • Monitoring to check temperature records of sample storage and see evidence of receipt at labs confirming conditions satisfactory in transit. |
| PK Analysis | Blood sample analysis | M | Yes. Invalid assay values. | • Sponsor (Trust) to confirm that assay method (Analytical Liquid Chromatography Mass Spectrometry (LC-MS)) has been validated.<br>• Assessment of laboratory undertaken (e.g. audit of facilities to confirm standards be adhered to (e.g. GCP Labs)<br>• The Laboratory are certified by Clinical Pathology Accreditation Standards for Medical Laboratories ISO 15189.<br>• Specialised laboratory experienced in Neonatal PK analysis and measuring small assay volumes<br>• Contract in place with laboratories as part of the CTA and Material Transfer Agreement<br>• Cross check the timing of source data using blood gas analyser times, arrival in the laboratory and ask phlebotomists to record the time they take scavenged | • Check laboratory facilities contracts in place and confirm assay validation report available. (could be done centrally) |

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| | | | | samples. | |
| | PK data analysis | M | None, provided the data are accurate. | • Specialist PK Laboratory (Paris) Accredited with PK Analysis experience in neonatal sampling (Trust sponsor has made an assessment) and Specialised statistical Modelling | |
| Other sample analysis | Microbiology analysis | L | None | • Standard operating procedure for testing faeces and MIC to Ciprofloxacin<br>• Minimum inhibitory concentration – based on EUCAST method and standards (European Committee on Antimicrobial Testing) | |
| | Biochemistry/ Haematology etc. | L | None. Use of hospital labs as per normal clinical practice | • No need for normal ranges for these to be filed in TMF. | |

## 5. Facilities, Equipment and Resources

| Area | Particular risk identified | Low Medium High | Concerns | Mitigation or Adaptation | Monitoring methods to address |
|---|---|---|---|---|---|
| Facilities | 2 recruiting sites, but complex follow up as neonates are transferred nearer home and thus 40 follow up sites for Pharmacovigilance reporting for 6 weeks – failure to obtain follow up info. | L | None regarding recruiting sites provided other risks mitigated. Facilities for the trial appropriate.<br><br>Yes. Collection of faeces samples and obtaining reliable safety information from 40 follow up sites | • Standard operating procedures are prepared for recruiting sites and separate ones for follow up sites.<br>• Recruiting Site assessment process in place. Site contracts and R&D approvals in place at recruiting sites, an induction programme is provided for the research team and trial tools provided.<br>• Follow up sites -. The R&D will take responsibility for GCP and CV at their site. A statement of responsibilities replaces a contract as this is simply data collection and a faeces sample.<br>• Principal Investigator at each site will report pharmacovigilance for up to 6 weeks<br>• R&D approvals and statement of responsibilities for follow up sites<br>• Accredited hospital laboratory are trained by the trial team and a dummy run of samples to test the systems. | Check contracts on TMF<br><br>Follow up sites are assessed remotely on receipt of R&D approval and acceptance of the role by a neonatal Principal Investigator<br>CLRN will check the Statement of Responsibilities |

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| | | | | • Follow up data is recorded in the CRF and the discharge letter is filed to cross check the incidence of adverse events | |
| Staff Training | Precision of samples and compliance with processes | H | Yes. Large clinical team involvement (200). Risk that proper process not followed and lack of precision in dosing and blood sample taking etc. | • Detailed SOPs<br>• Formal training of clinical staff 200+ per site in protocol and trial specific SOPs<br>• The research team will hand hold the procedures and speak to the nurse on duty each shift.<br>• GCP training for Investigators and those with substantial roles (recruiting and pharmacovigilance reporting)<br>• Training delivered to the clinical teams by staff who are GCP trained Training packs for the bedside<br>• The clinical team are not GCP trained but will be trained in the tasks they are required to do consistent with GCP standards.<br>• Formal Trial Training presentation/initiation visit to explain trial tools at recruiting sites.<br>• Ensure SOP are consistent with the sites clinical practice.<br>• Training log for day/night staff<br>• Delegation log – PI approves research team are appropriately trained prior to involvement in trial activities. | • Review of training and delegation logs. Cross check trained individuals taking consent, dosing and blood sampling. |
| Follow Up | Loss to Follow up - babies may be transferred to any hospital within 200 miles during follow up | L | Yes. Loss of follow up data and PV data. | • Principal Investigator at follow up site with GCP training is required for safety reporting during 6 week follow up<br>• Generic SSI used for R&D approvals for 40 sites administered by the Comprehensive Local Research Network<br>• Standard operating procedure specifically for follow up sites including pharmacovigilance reporting and transfer of Category B faeces samples<br>• Follow up with 'discharge letter from each site.' | • Collation and checks of approvals (central monitoring). Maintenance of approved site list. Monitoring to check follow ups at approved sites (can be done centrally). |

| 6. Documentation, Governance and GCP Compliance | | | | | |
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| **Area** | **Particular risk identified** | **Low Medium High** | **Concerns** | **Mitigation or Adaptation** | **Monitoring methods to address** |
| TMF | Lack of documentation to reconstruct trial and confirm compliance with CT regulations, the protection of subject's rights/well being/safety and the reliability of the trial results. | L | None. | • TMF centralised at the Chief Investigator recruiting sites (ICH GCP Section 8.2) and a Site Trial File at the other recruiting site containing essential documents<br>• CV and GCP certificates are filed centrally at the NIHR site for the investigating team, which are accessible long term.<br>• Retention of TMF 21 years supporting Paediatric Investigation Plan (required for neonates growth and development issues) stored by NHS approved archiving (ICH GCP Compliant).<br>• Trial files are not required at non recruiting follow up sites<br>• The Statement of Responsibilities (section 1) states that a trial file is not required at the non recruiting follow up sites as approved by the R&D Forum | • Monitor to review recruiting site TMF at least once during trial conduct to ensure adequately maintained.<br>• Monitor to confirm suitable archiving arrangements for all TMF |
| Monitoring | Inadequate monitoring.<br><br>1) Regulatory<br>2) Source data<br>3) Data reliability | <br><br>L<br>L<br>H | Yes. Lack of monitoring appropriately could result in non-compliance and inaccurate data for the PK objective.<br><br>Minimal risk of loss to follow up data | • Recruiting sites: Sponsor (Trust R&D Department) monitor the study and undertake source data verification (SDV) based on risks identified – process to be documented in a Monitoring Plan.<br>• Compliance with trial SOP are assessed daily by the researcher during the study and on completion of the case report forms by checking sample times /infusions/ or protocol deviations. Serious breaches are risk managed by detailed standard operating procedures, detailed trial tools and staff training.<br>• Serious Breaches that affect the safety of the subject or the scientific integrity of the trial are reported to the Sponsor (Trust R&D Policy) as per Regulation 29A [8] A root-cause analysis, corrective and preventive actions will be undertaken. If defined as a serious breach by the Sponsor (Trust) it will be reported to the MHRA within 7 days.<br>• Monitoring is not required at follow up sites – data will be recorded according to the SOP and further information | • There will be a need for onsite monitoring at the recruiting sites with targeted Source Data Verification. Some central review of documentation may be possible.<br>• The monitor will design tools to assess 1) Governance 2) Source data 3) data reliability for SOP compliance in the ward and laboratory. |

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| | | | | requested from the Principal Investigator at the site when required. | |
| Sponsors | Inadequate oversight. | | None, but potential if there is a lack of clear sponsor responsibilities that could result in non-compliance. | • Trial Sponsored by Liverpool Women's NHS FT and University of Liverpool.<br>• Sponsor and Co-Sponsor roles and responsibilities will be defined & Co-sponsorship contract in place.<br>• An Agreement exists for the provision of Research Management Services between the University and Trust detailing the clinical research management of the study by the Trust (as the Co-ordinating Centre).<br>• All trial procedures undertaken by both hospital sites and contracts or agreements in place. | • Check contracts in place and they cover all responsibilities under the legislation. |

## Approval

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|---|---|---|---|---|---|---|---|
| **Chief Investigator:** | | **Date:** | | **Sponsor:** | | **Date:** | |
| | | | | **Sponsor:** | | **Date:** | |

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